TEXAS A&M UNIVERSITY

AgriLIFE RESEARCH
Texas A&M System

Improving Life Through Science and Technology

Rewiring Photosynthesis for Terpene Production

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Texas A&M University

In cooperation with:
SynShark LLC

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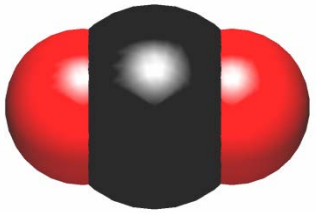
Challenges for Sustainable Fuels and Chemicals – a Carbon Perspective

Sunlight

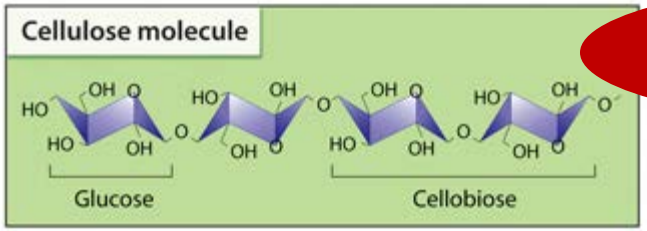
Water



Carbon Dioxide

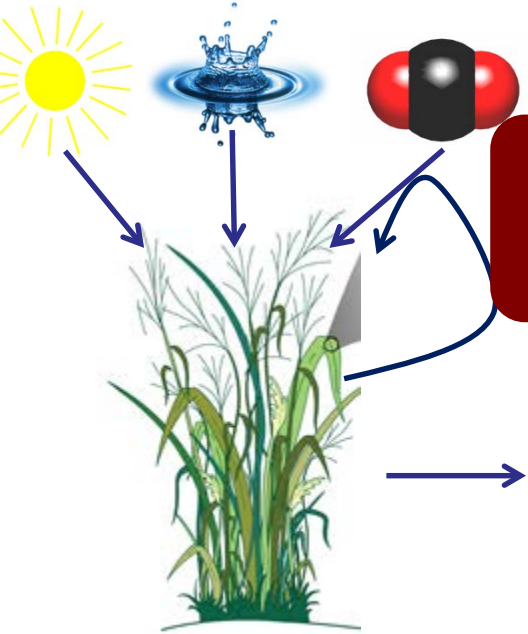
Photosynthesis
Photorespiration

Enhancing Carbon Flux for Target Products

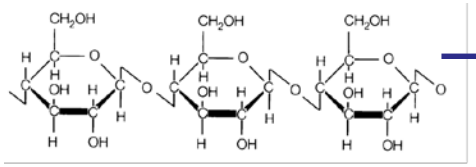


Lignin and Other Waste

Roadmap for Complete Carbon Usage and Sustainable Biofuel and Bioproducts

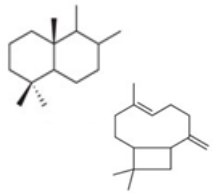


Redirecting Photosynthetic Carbon Output



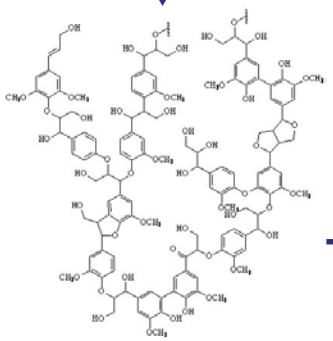
Bioconversion into Fuels & Chemicals

Cyanobacteria to Capture and Utilize CO_2 for Hydrocarbon



Extracted or Pretreated Biomass

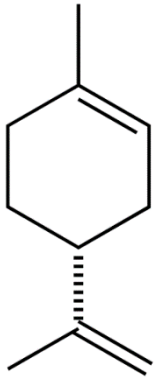
Terpenoid as Drop-in Fuels and High Value Products



Bioconversion into Fuels and Chemicals; Fractionation for Valued Products

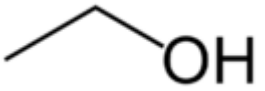
Terpenes for Fuels and Chemicals

- Thermophysical and thermochemical properties of target fuel molecules
  - Energy Density
  - Cloudy Points
  - Others
  - ‘Drop in’ biofuels
  - Minimal hydrogen consumption during cracking
- Diverse product stream amenable to different markets – squalene, astaxanthin, taxadiene, etc.

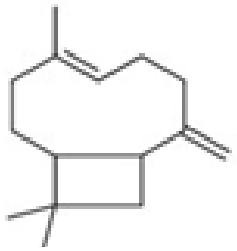


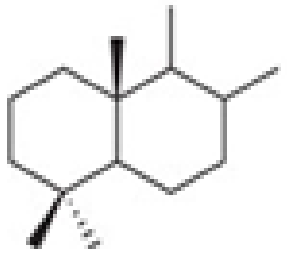
Energy Density
45 MJ/Kg
38 MJ/L

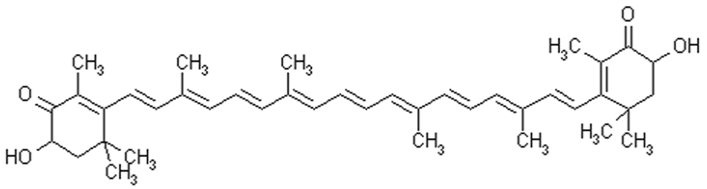
Ethanol



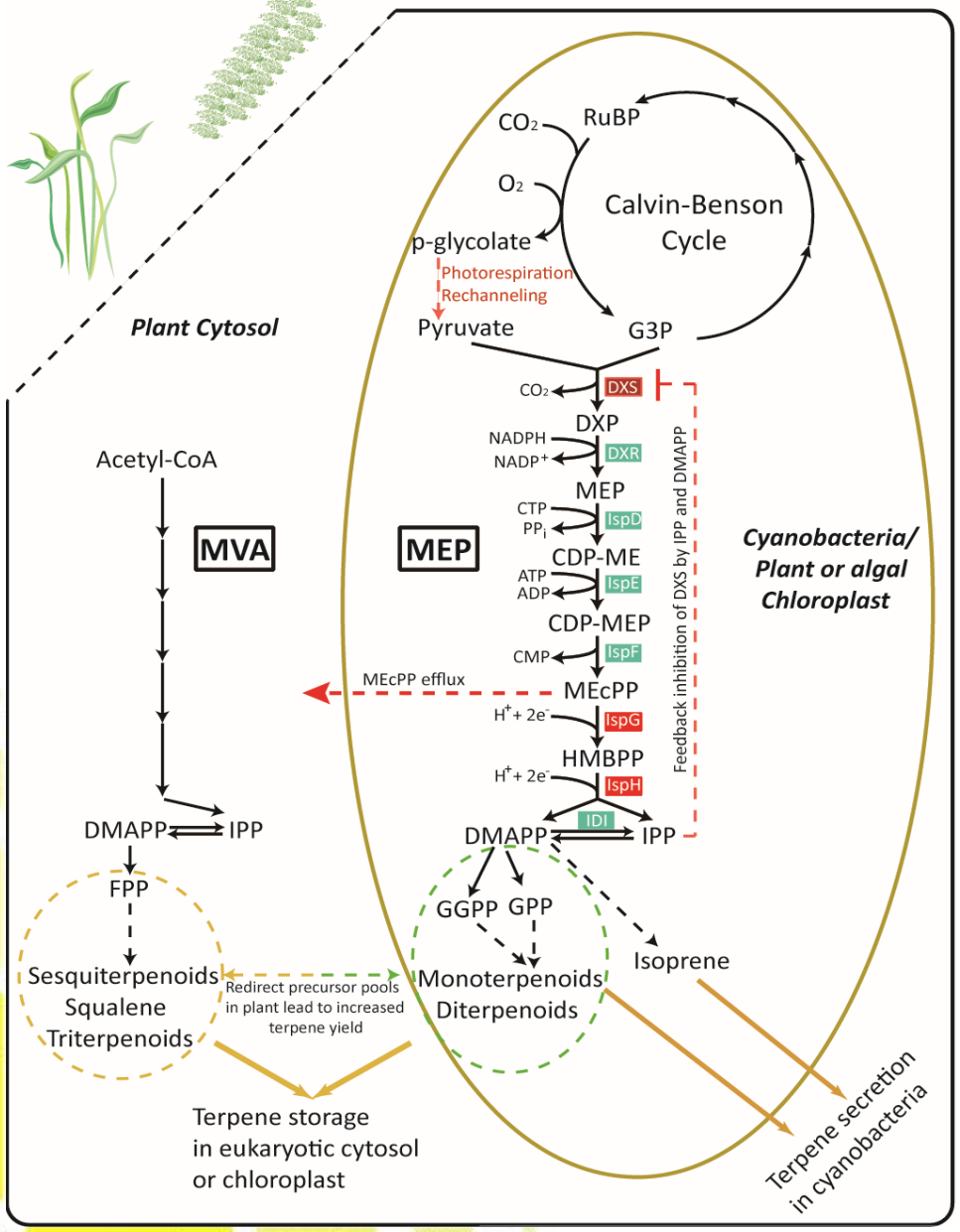
Energy Density
30 MJ/Kg
24 MJ/L





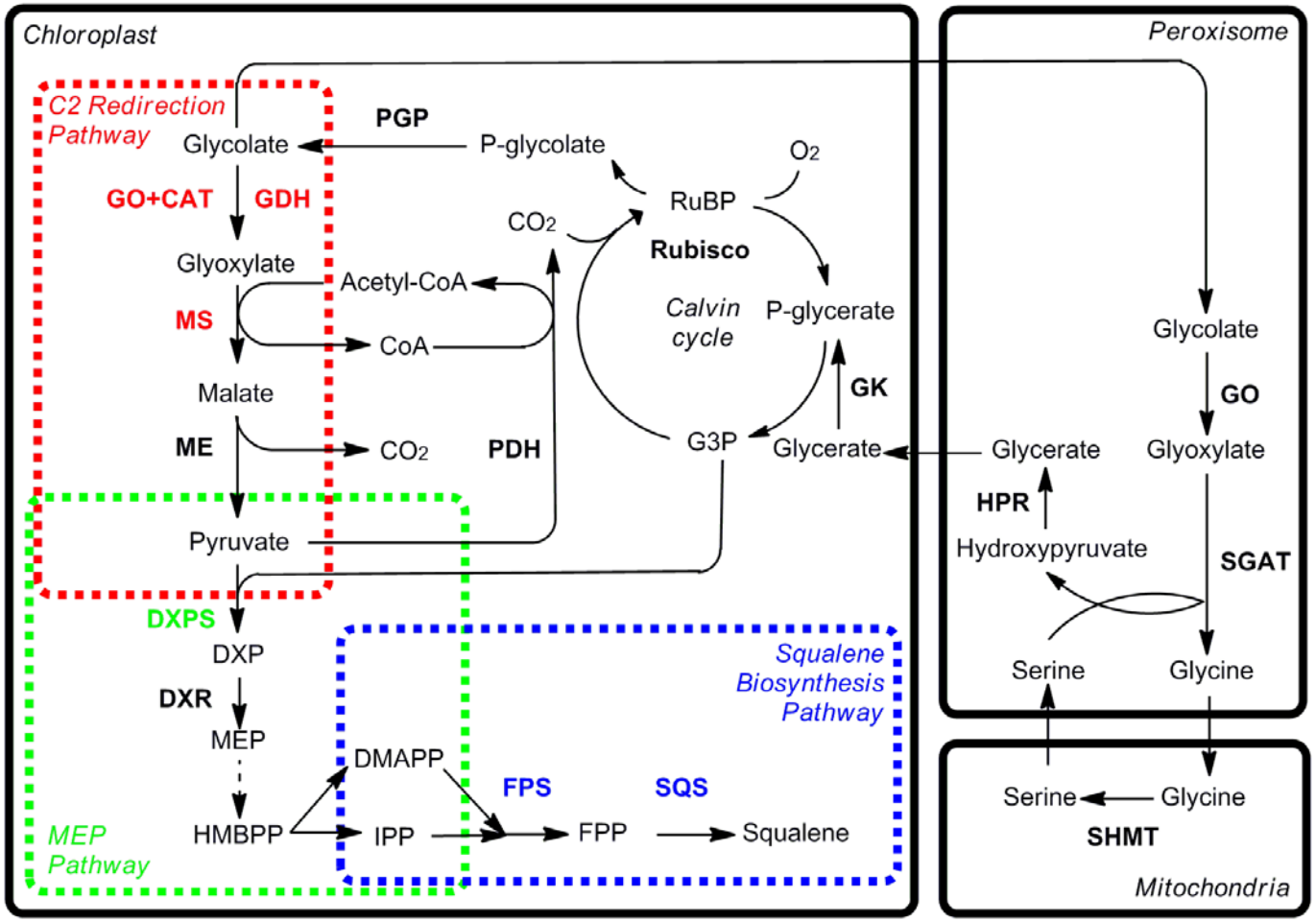


Scientific Challenges



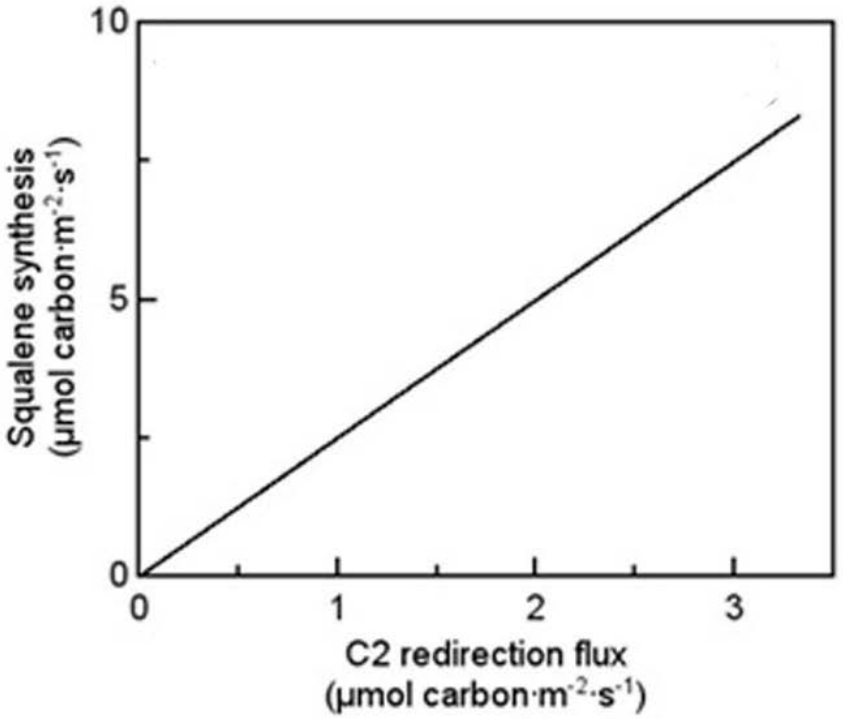
- **Scientifically, perfect unconventional sink!**
- **Low carbon partition**
- **Limited by carbon fixation in photosynthesis**
- **Limited by extensive feedback and storage of target compounds.**

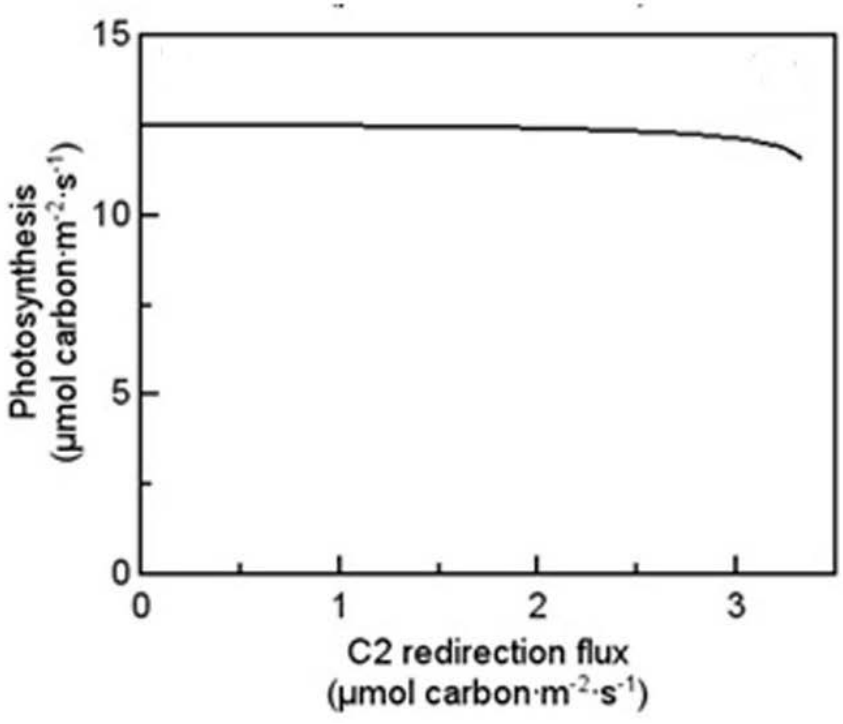
Rewiring Photorespiration Products



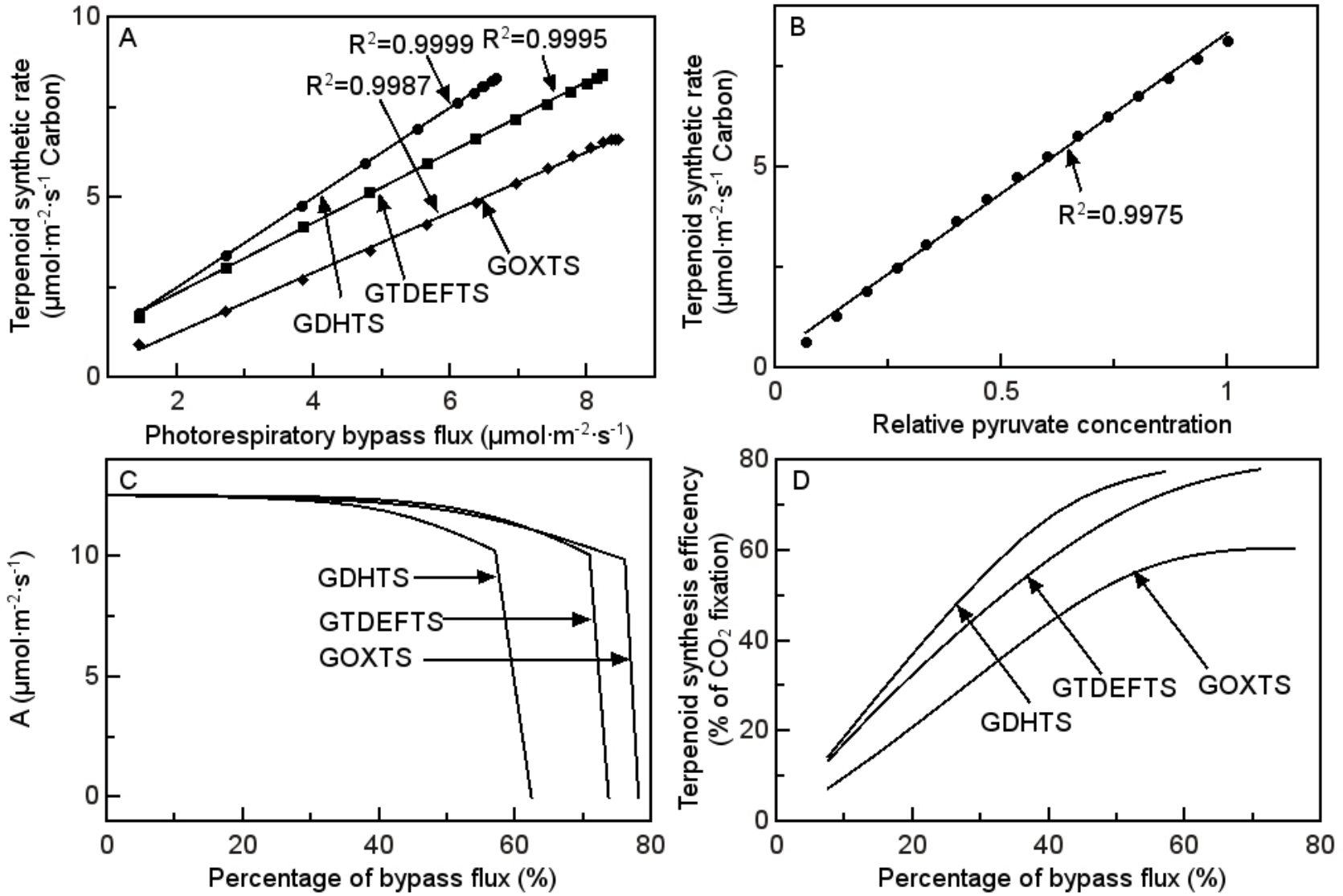
CAT, catalase; **DXPS**, 1-deoxy-D-xylulose 5-phosphate synthase; **DXR**, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; **FPS**, farnesyl diphosphate synthase; **GDH**, glycolate dehydrogenase; **GK** glycerate kinase; **GO**, glycolate oxidase; **HPR**, hydroxypyruvate; **ME**, malic enzyme; **MS**, malate synthase; **PGP**, phosphoglycolate phosphatase; **SGAT**, serine-glutamate aminotransferase; **SHMT**, serine hydroxymethyl transferase; **SQS**, squalene synthase; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; FPP, farnesyl diphosphate; IPP, isopentenyl diphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate.

Computational Modeling Indicated that C2 Redirection Could Increase Terpene Yield by Providing More Pyruvate



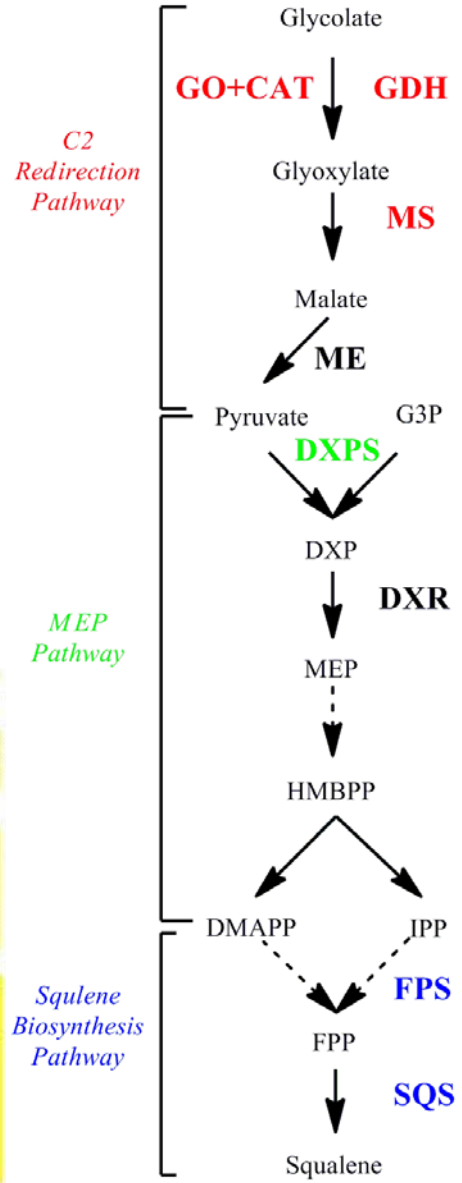


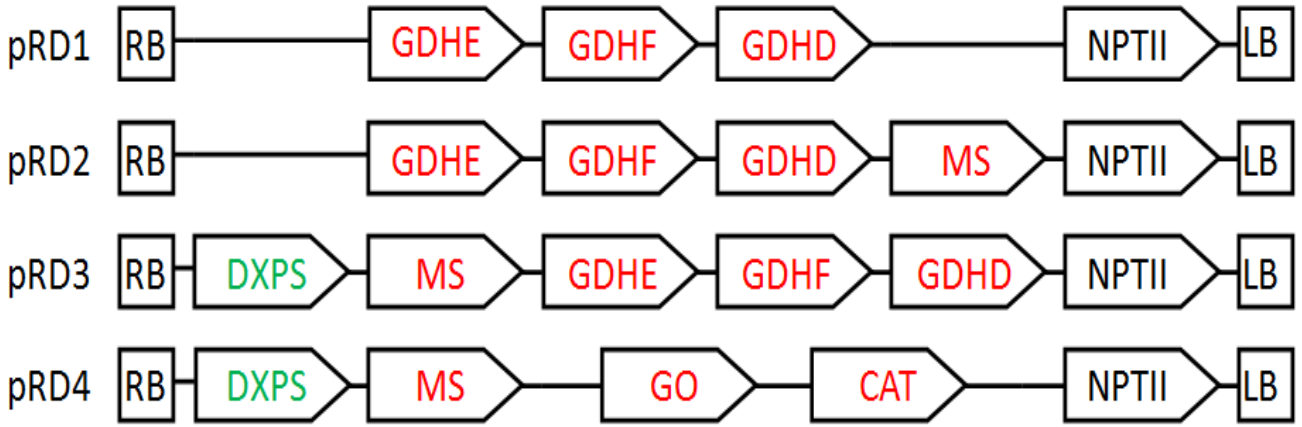
***Photosynthesis rate will not be significantly impacted with our design to channel carbon to high value products**



The effect of photorespiratory flux through the bypass on terpene synthesis (A), the effect of available pyruvate concentration on terpene synthesis (B), the effect of the percentage of photorespiratory flux through the bypass on photosynthesis (C), and the effect of the percentage of photorespiratory flux through the bypass on terpene synthesis efficiency (D) of different engineered terpene synthesis strategies.

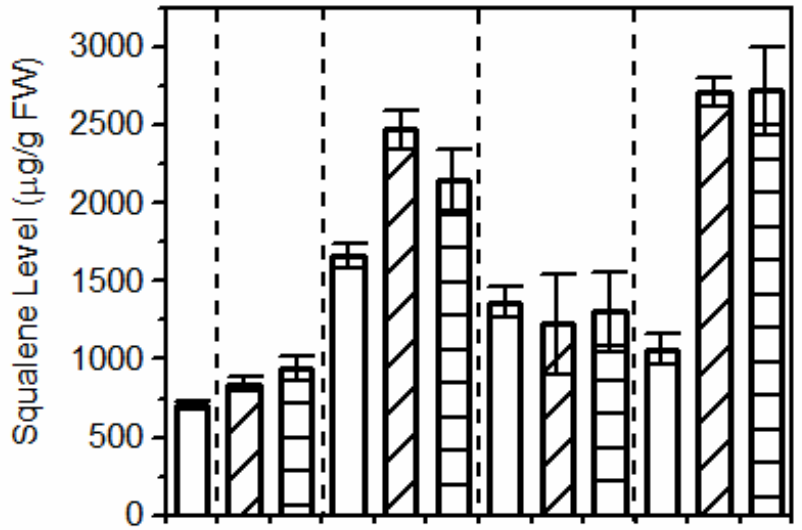
Pathway Design and Vectors

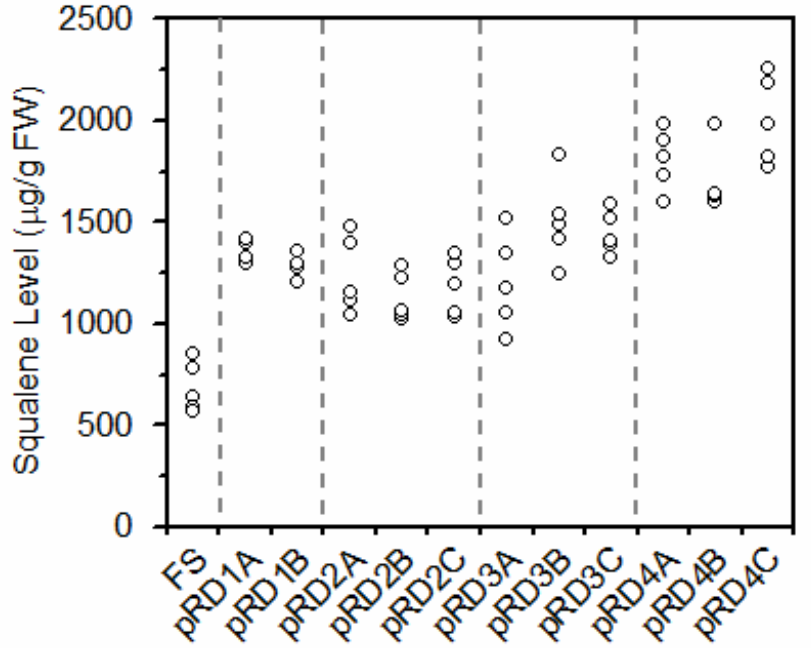




- pRD1=GDH only (partial by-pass)
- pRD2=GDH+MS (complete by-pass)
- pRD3=GDH+MS+DXP
- pRD4=GO+CAT+MS+DXP
- FS parental line= FPS+SQS

C2 Redirection Increases Squalene Production by 2 to 4 Folds

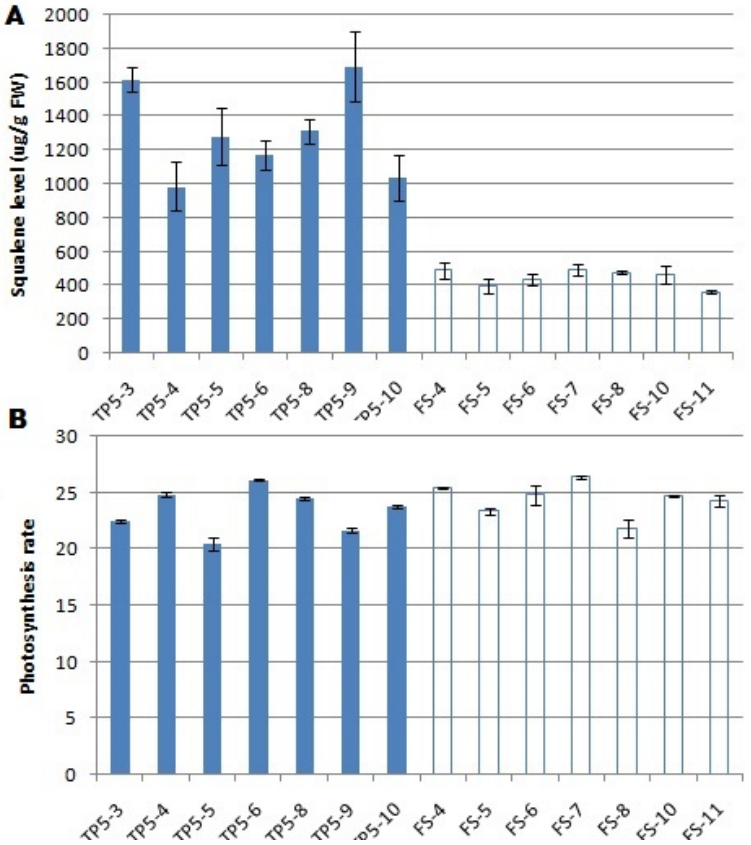




- pRD1=GDH
- pRD2=GDH+MS
- pRD3=GDH+MS+DXP
- pRD4=GO+CAT+MS+DXP
- FS parental line= FPS+SQS

Initial Field Trial Confirmed Squalene Increases

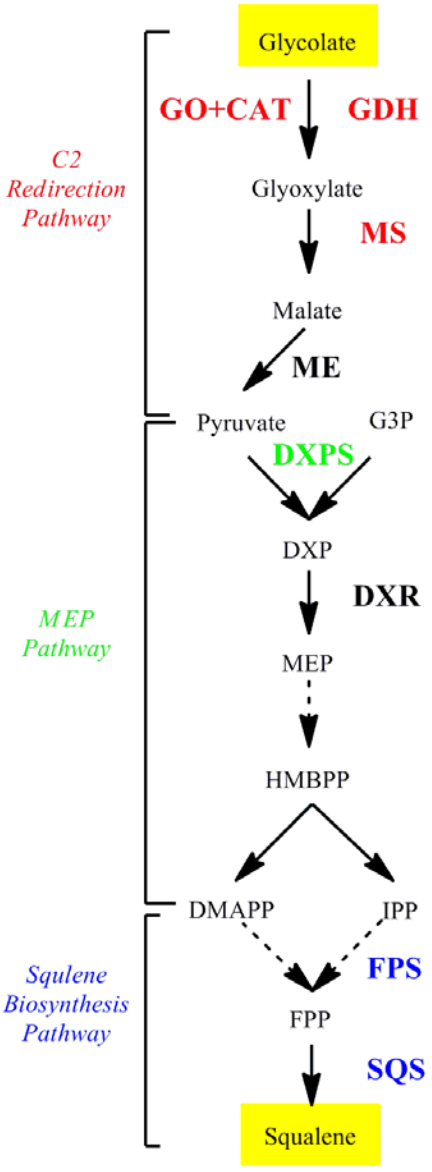
	Plant Height	Leaf No.	Leaf Length	Leaf width	Stem girth	Average internode length
1068	76.78±19.06	22.73±4.18	70.33±6.03	29.42±4.75	4.32±1.92	3.3±0.55
pRD4	50.55±9.03**	19.33±2.69*	57.5±8.08**	31.77±3.39	2.51±0.67**	2.58±0.31**
HG2	60.01±11.29**	20.13±2.67*	63.09±5.17**	33.41±3.37	3.69±0.8**	2.92±0.41*
HG4	55.32±9.35**	20.93±2.43*	58.3±8.41**	31.87±3.71	2.6±0.56**	2.59±0.34**



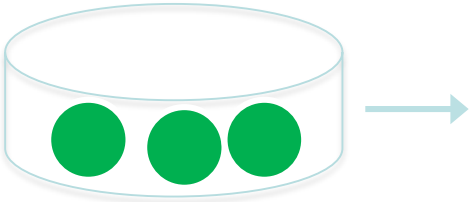
2017 & beyond: hundreds of acres

From 2017, SynShark will be able to expand both at the Short farms, where hundreds of plant- able acres are available, or through full commercialization of its seedlings.

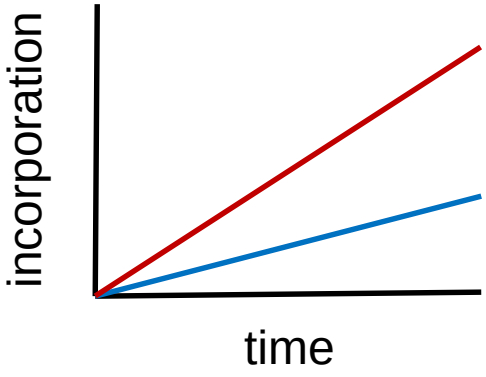
C14-Glycolate Feeding Assay to Evaluate Carbon Flux



Pulse-label with radiolabeled glycolate



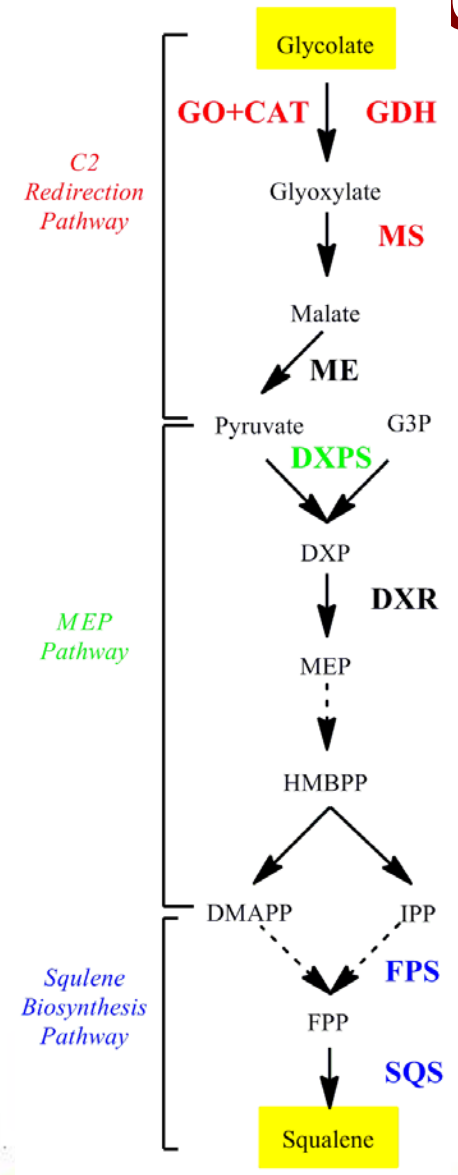
Collect samples at specific time points (0, 12, 24 hrs) and determine the amount of label incorporated into squalene

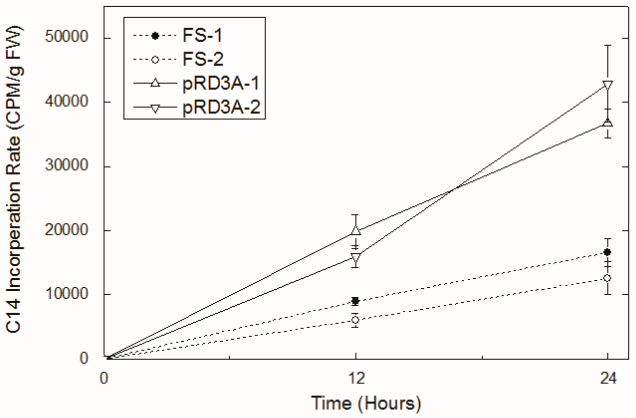


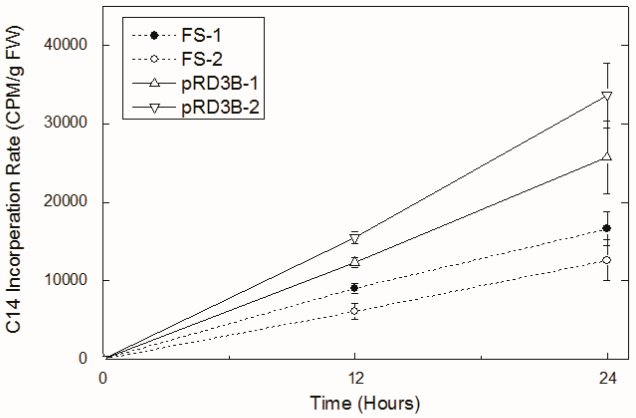
pRD transgenic tobacco

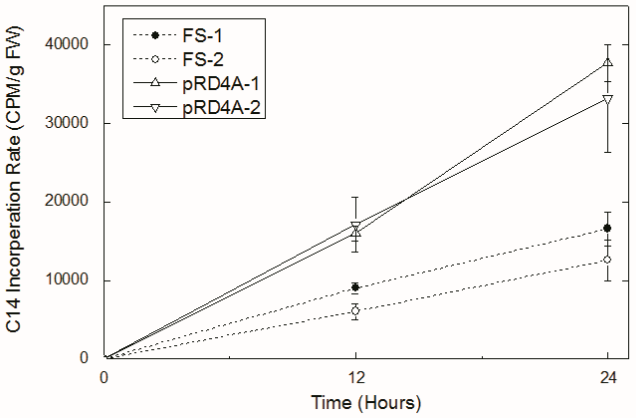
FS parental line

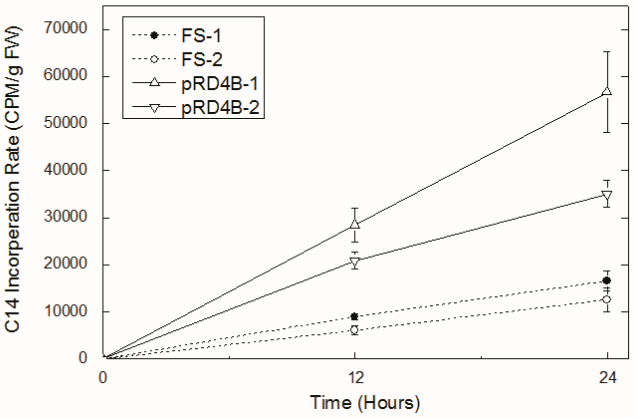
C2 Redirection Enhances Incorporation of Glycolate into Squalene



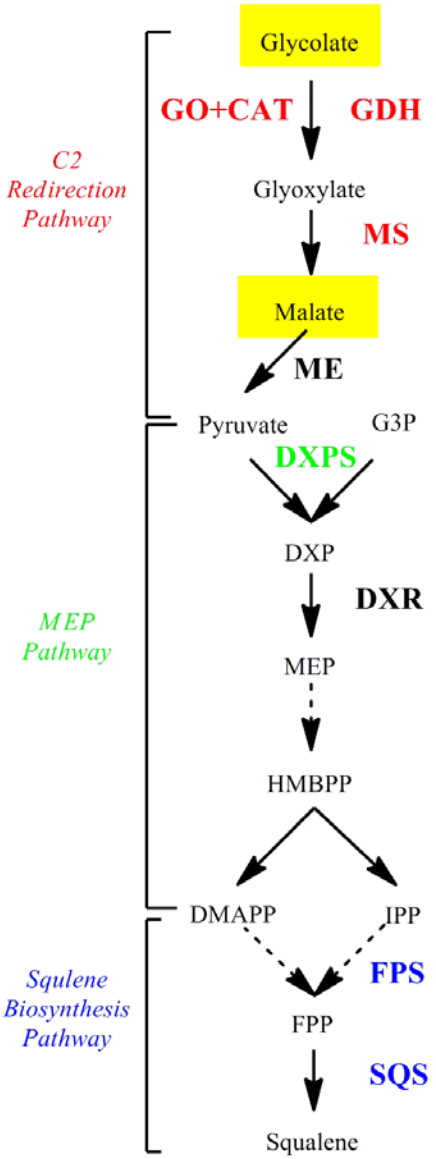


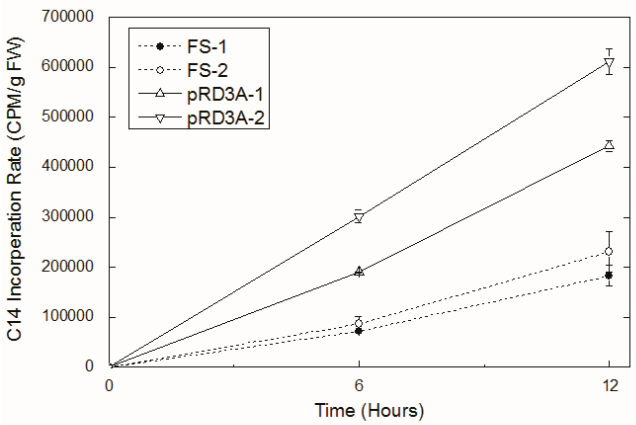


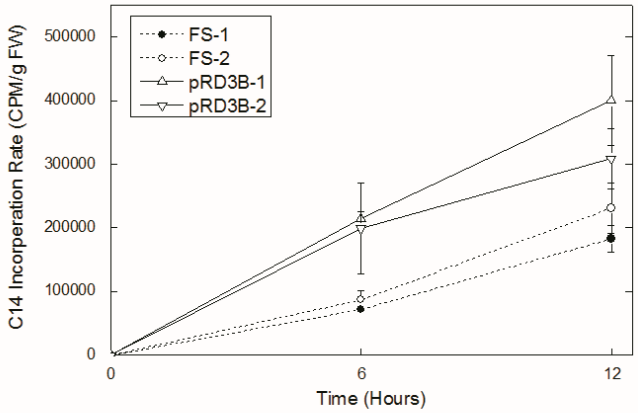


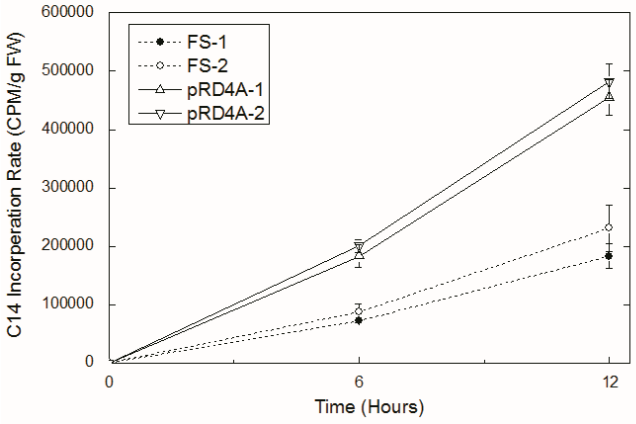


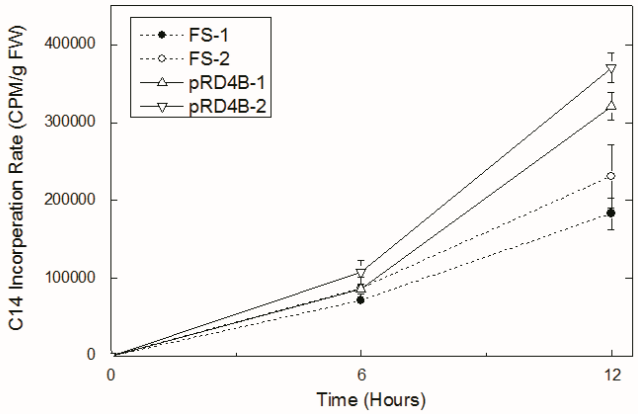
C2 Redirection Enhanced Incorporation of Glycolate into Malate



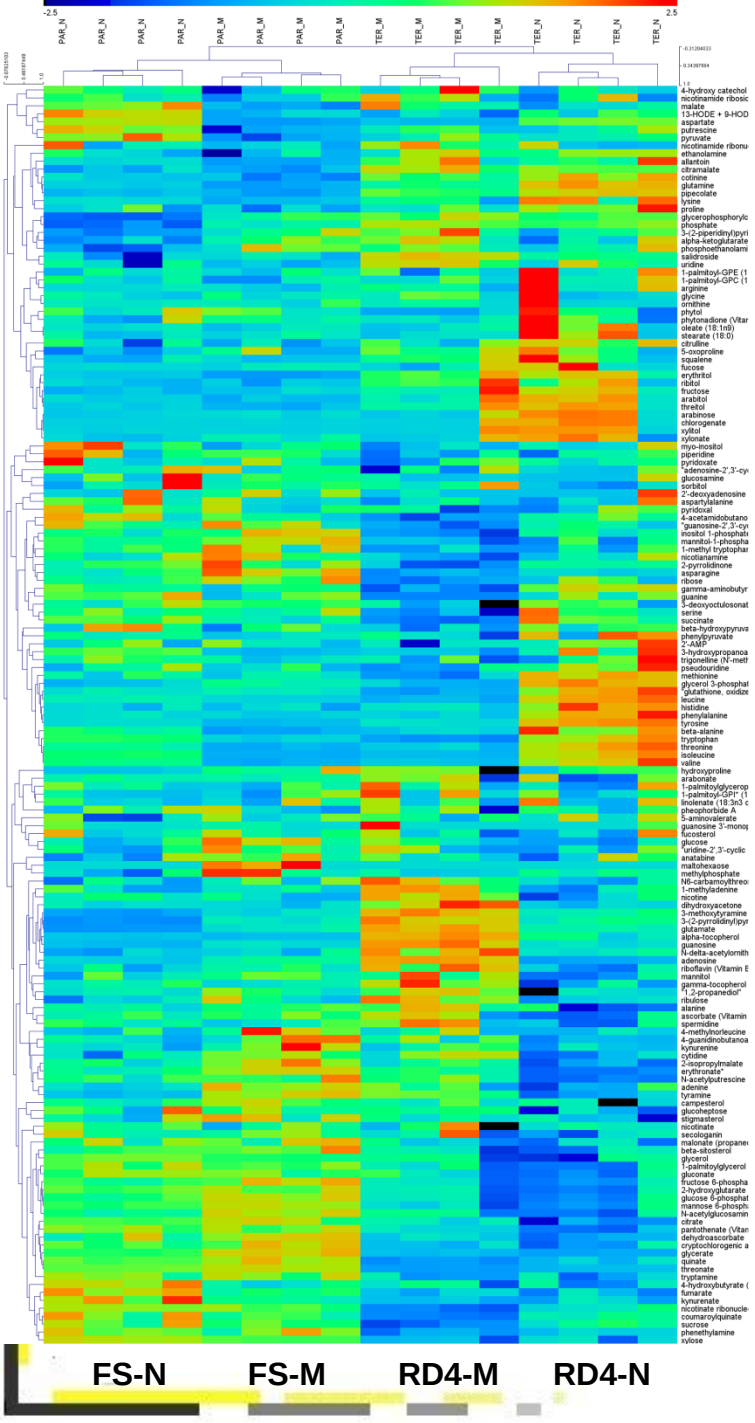








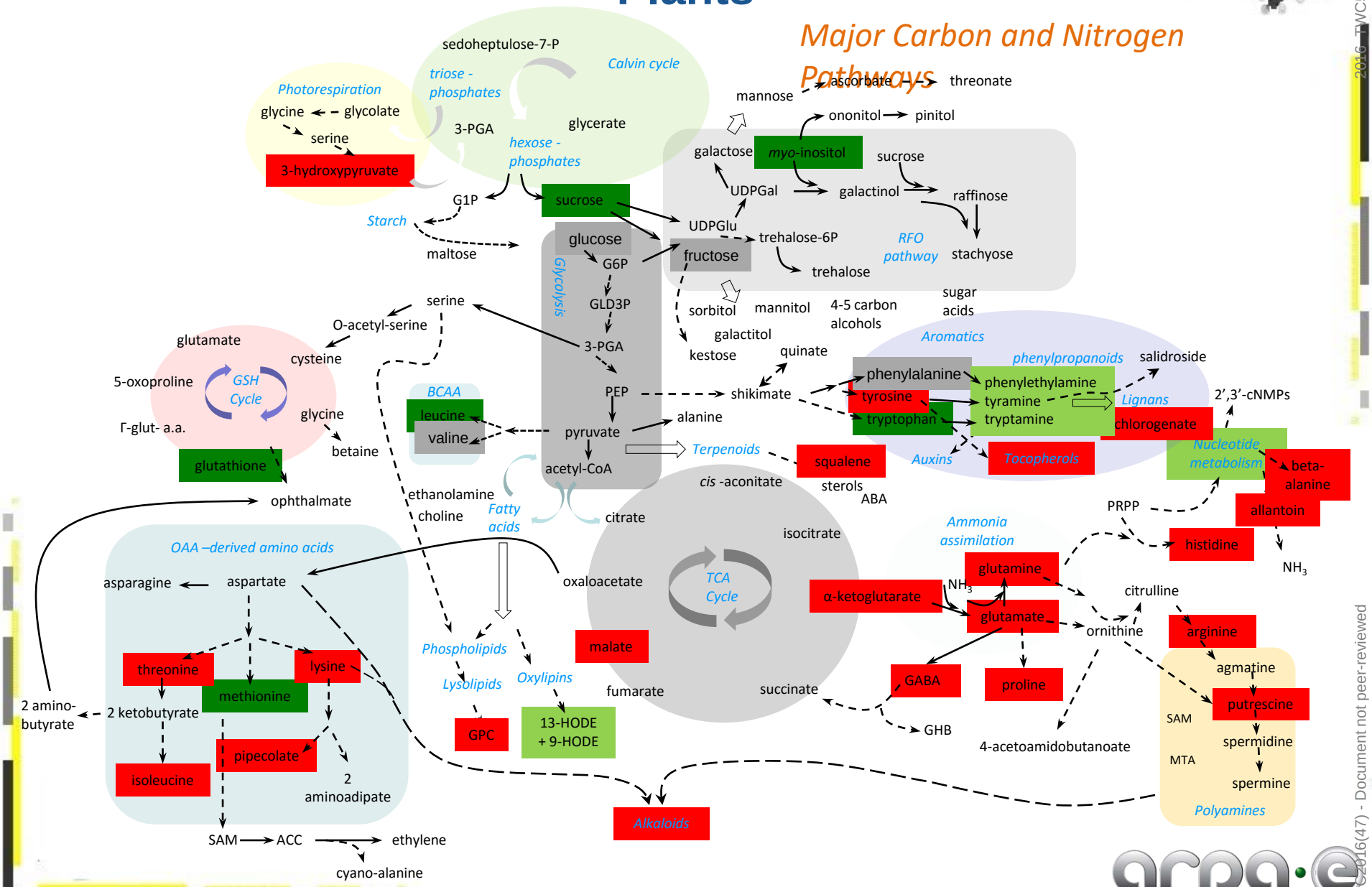
Global Metabolite Profiling to Evaluate Carbon Output



- GC-MS and UPLC-MS/MS platforms
- 162 metabolites identified
- 4 repeats of each sample clustered together

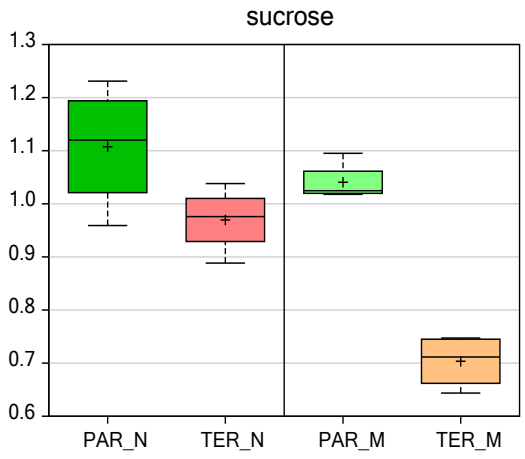
FS-N: FS plant samples collected during night time
FS-M: FS plants samples collected during daytime
RD4-N: RD4 plant samples collected during night time
RD4-M: RD4 plant samples collected during the daytime

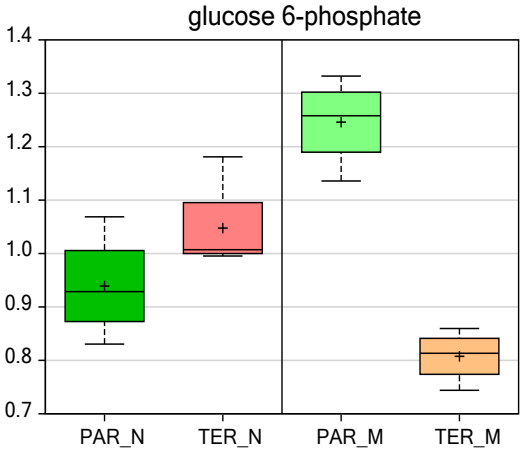
The Overview of Metabolite Changes in C2 Redirection Plants

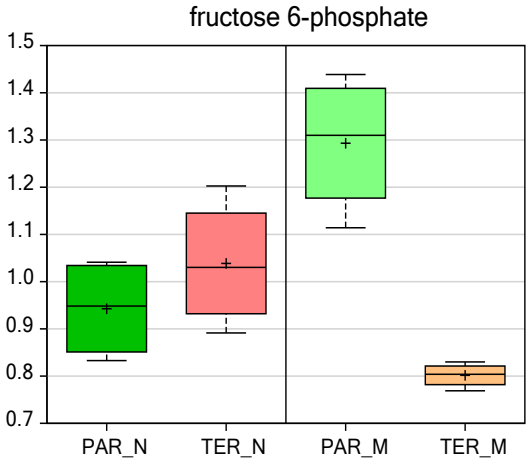


Decreased Carbon Partition to Sucrose and Starch Biosynthesis

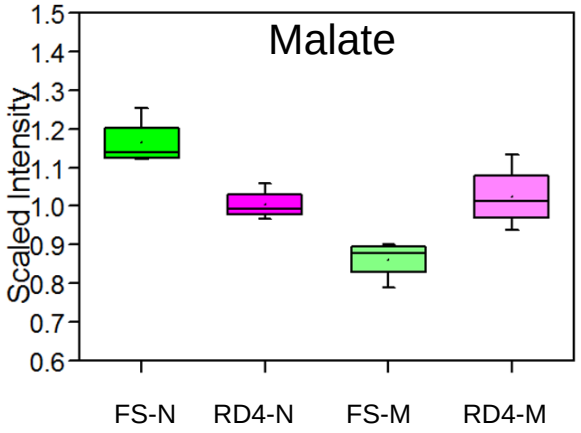
- Lowered pools of sucrose, starch intermediates, and hexose phosphates in morning, along with higher pyruvate and hexoses, suggests a shortage of carbon for energy production in pTp5 samples relative to G1 in the light phase.
- There are ~35% decrease in sucrose, 33% decrease in G6P, and 37% decrease in F6P. The decrease in the sucrose, G6P and F6P are very consistent, indicating a significant carbon repartition process.

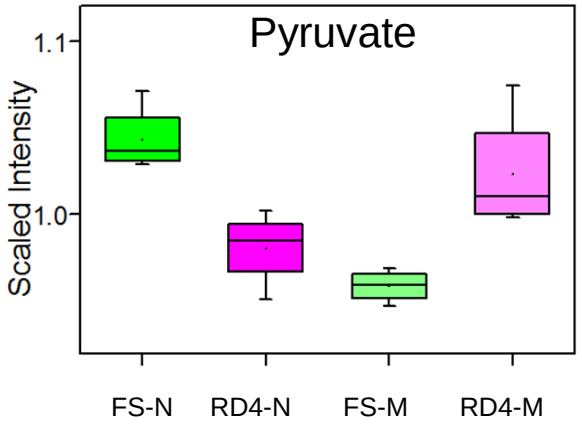


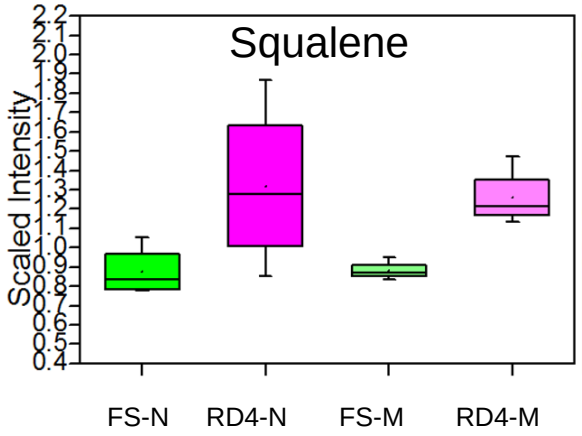


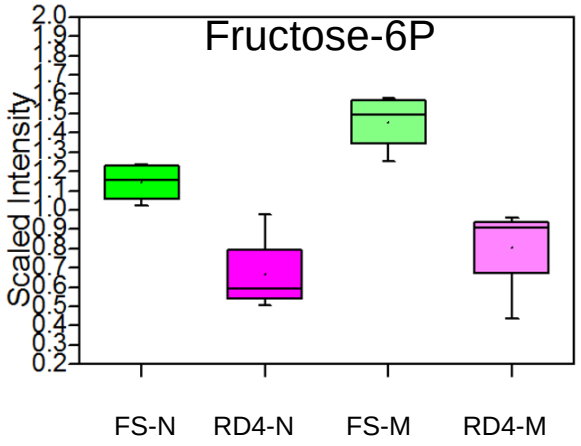


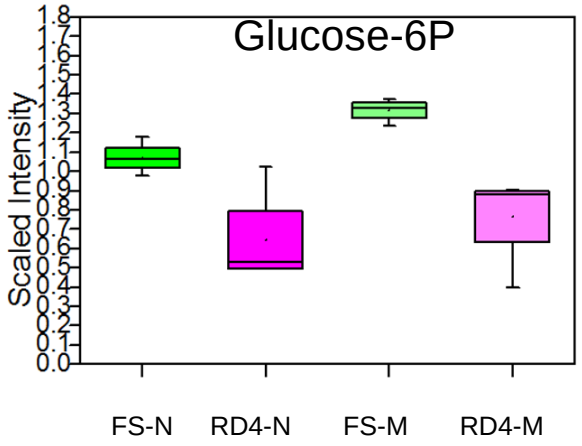
Relative Abundance of Key Metabolites

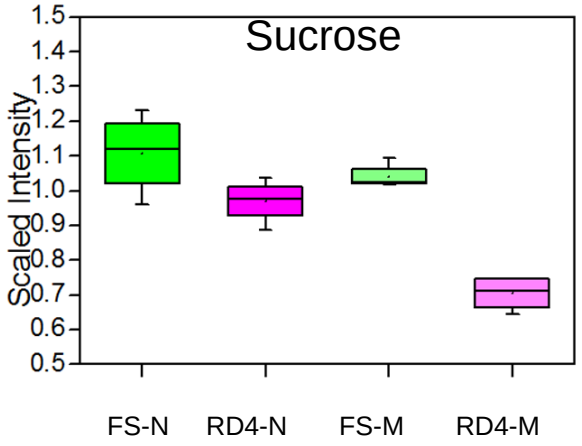




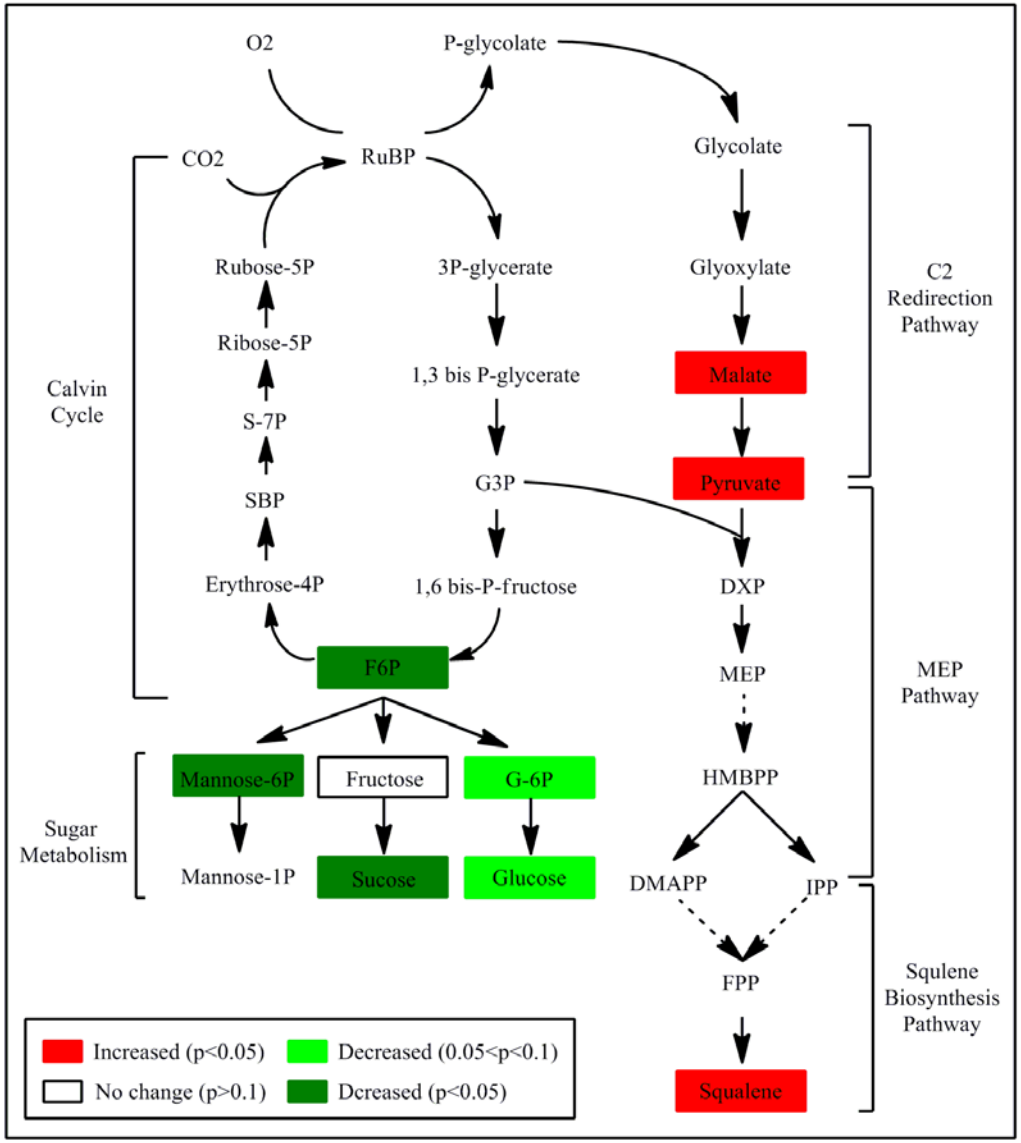








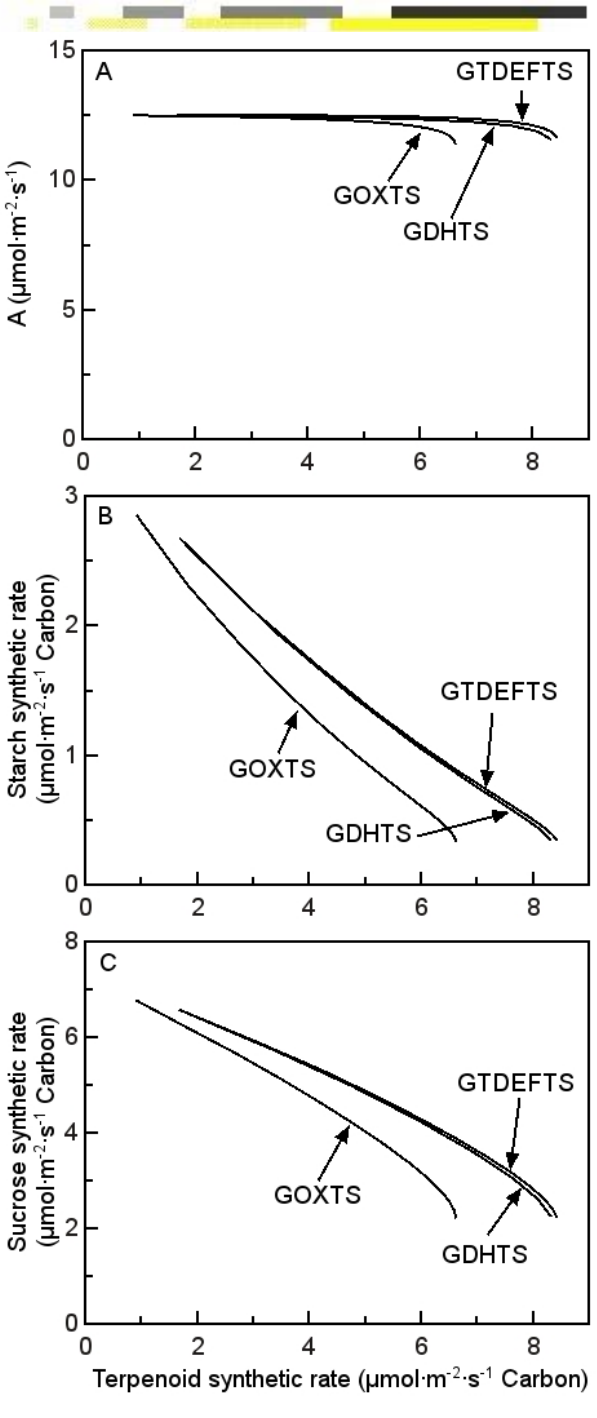
C2 Redirection Leads to a Significant Carbon Repartition



The mathematical model predicts an unchanged photosynthesis, but significantly decreased sucrose and starch output.

The modeling data fits very well into experimental data in three aspects:

- **Increased Terpene**
- **Decreased Sucrose and Starch Biosynthesis**
- **No Significant Changes in Photosynthesis**

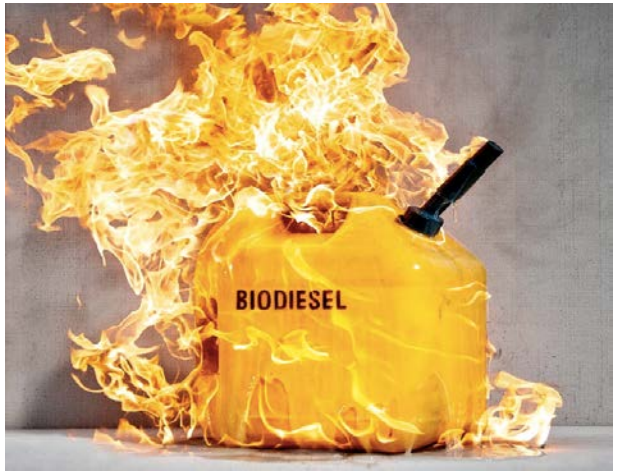


Summary

- C2 redirection was designed to enhance terpene yield and to achieve 2.7 mg/g FW of squalene, which is about 2-4 fold increases from terpene engineering only.
- A C14 labelling assay verified that a functional C2 redirection pathway increases glycolate flux malate and squalene.
- Metabolomics data also indicated a significant carbon repartition from sugar metabolism to terpene biosynthesis resulted from the C2 redirection.
- The combination of C2 redirection with photosynthesis acceleration could further increase terpene yield.
- This research established a novel approach to produce high level of terpenes toward fuels, chemicals and pharmaceuticals in plants. More importantly, it indicated that synergy between photosynthesis and downstream engineering is crucial for photosynthetic production of terpene and other products.
- Other strategies including C5 redirection, C3/C6 redistribution, synthetic droplet to enhance terpene storage are on-going.

Commercialization with SynShark

Nature's substitute for scarce resources



At $1/liter vs $30/liter retail, the fuel market requires either a major tax or subsidy to support the real development of effective biofuels for transportation. ARPA-E studies, as well as the Company's, suggest that tobacco to squalene dry yield would need to reach at least 20% to be price competitive, a long shot considering the 2013 record yield of 0.6%. At the formation of SynShark, the Company's management pivoted the strategy away from fuels and into the higher margin specialty chemicals sector,

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Squalene is a natural element found within the human sebum. This is the skin's organic way to coat and protect skin from environmental aggressors, pollution, and free radicals that enter the epidermis and create issues with collagen and elastin.

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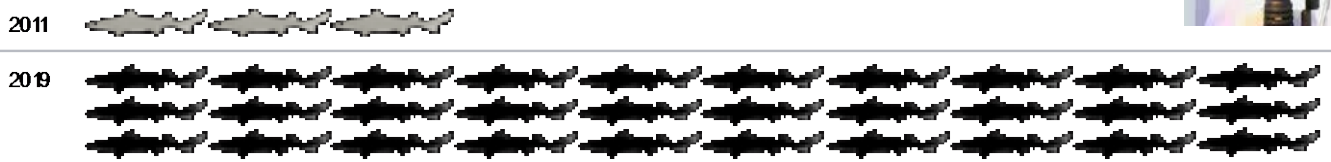
Squalane, derived from the hydrogenation of squalene, is also naturally present in human skin, but begins to decrease rapidly after the age of 25. Topically, this stable element boosts cell regeneration and oxidation.

Unsustainable catch: by 2019 over 30M sharks would be needed to match market demand



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