

Analyzing Tobacco Metabolism by NMR for Biofuel Production



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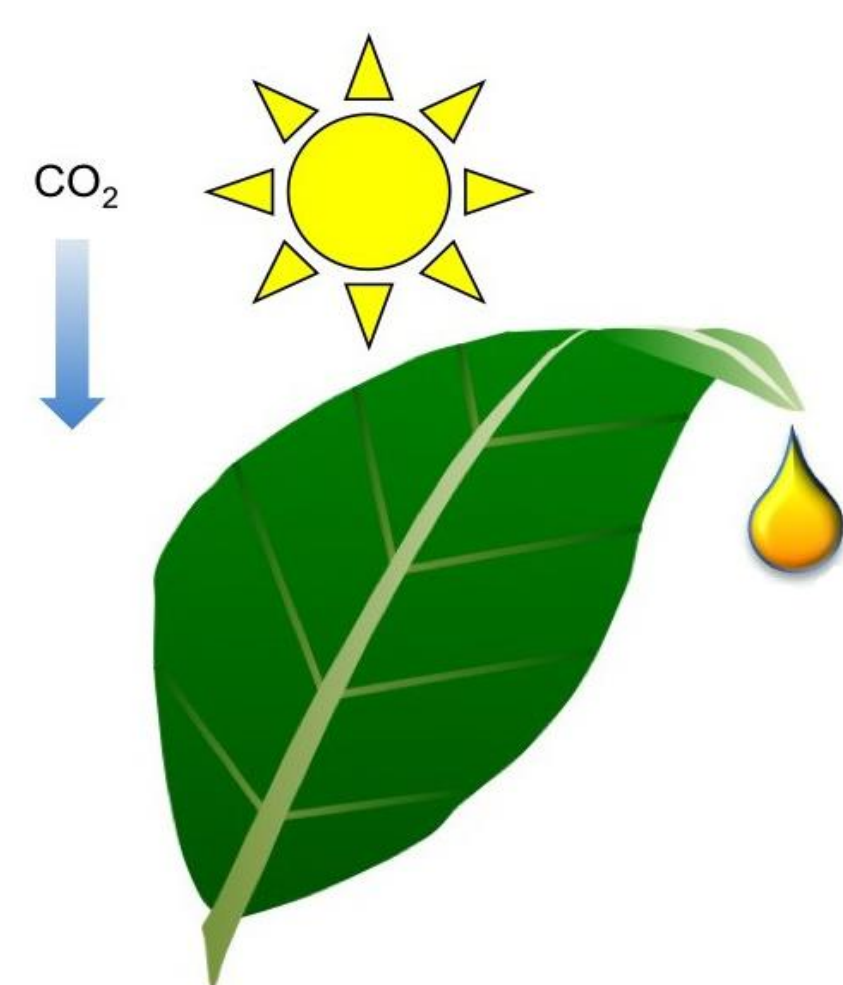


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Abstract

Our ultimate end goal is to take sunlight and CO₂ and produce biofuels by means of the tobacco plant's metabolism. My part in this goal is to give the biggest window into the metabolism of the plant by means of Carbon-13 labeling and NMR analysis. This is needed so that geneticists within The Jansson Group (LBNL) can modify plants and monitor the effects of those genetic changes. The way we accomplished this was to extract as many of the metabolites as we could using a 1:1 Methanol Water extraction method, then analyze and identify those metabolites by NMR. We also completed experiments to determine the time window of the production of metabolites in the Calvin and citric acid cycles by introducing only carbon-13 into the plant through the use of labeled ¹³CO₂. This allowed us to see metabolites build up over time by means of the carbon-13 NMR HSQC and compare the spectra according to how long the labeled carbon 13 was allowed to build up. In the end we were able to give the geneticists a better view into the tobacco metabolism so that we can make tobacco a viable biofuel producer through the means of genetic manipulation.

Sunlight and CO₂ to Biofuel via Tobacco

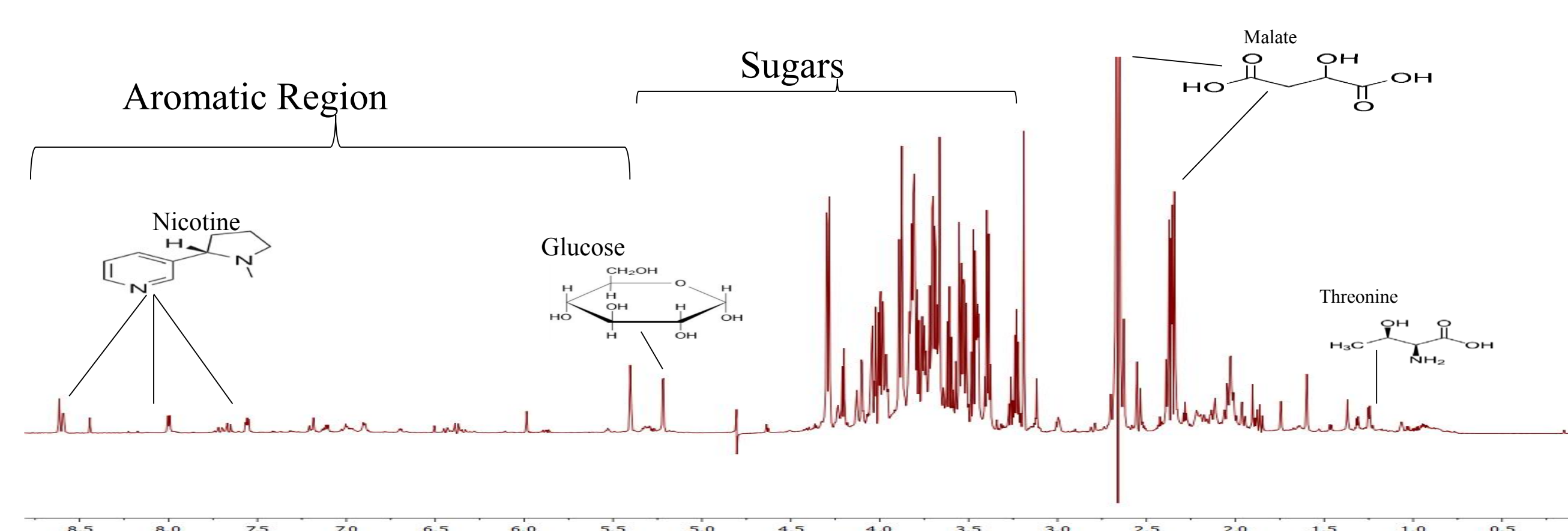


Why Tobacco Biofuel?

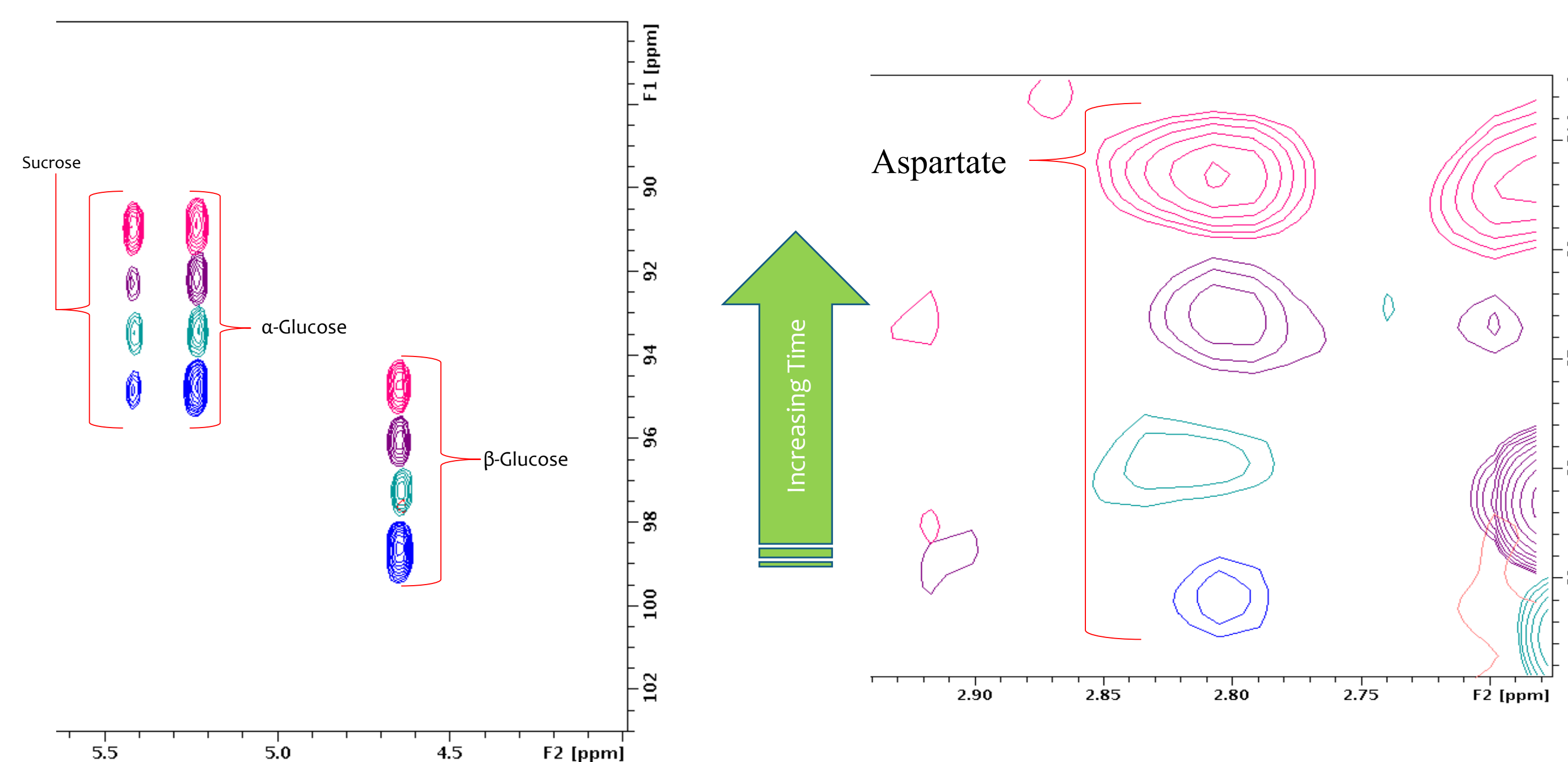
- Current fossil fuel supplies are diminishing while demand continues to grow
- A renewable energy source can help improve the economy by creating production based jobs
- Biofuels can reduce greenhouse gas emissions
- Some current biofuels use edible crops to create fuel
- The tobacco plant is not part of our food supply
- Infrastructure for tobacco already exists on a large scale worldwide
- Tobacco is easily amenable to genetic manipulation
- Areas where tobacco is already grown are usually not suitable for growth of food crops

NMR Data

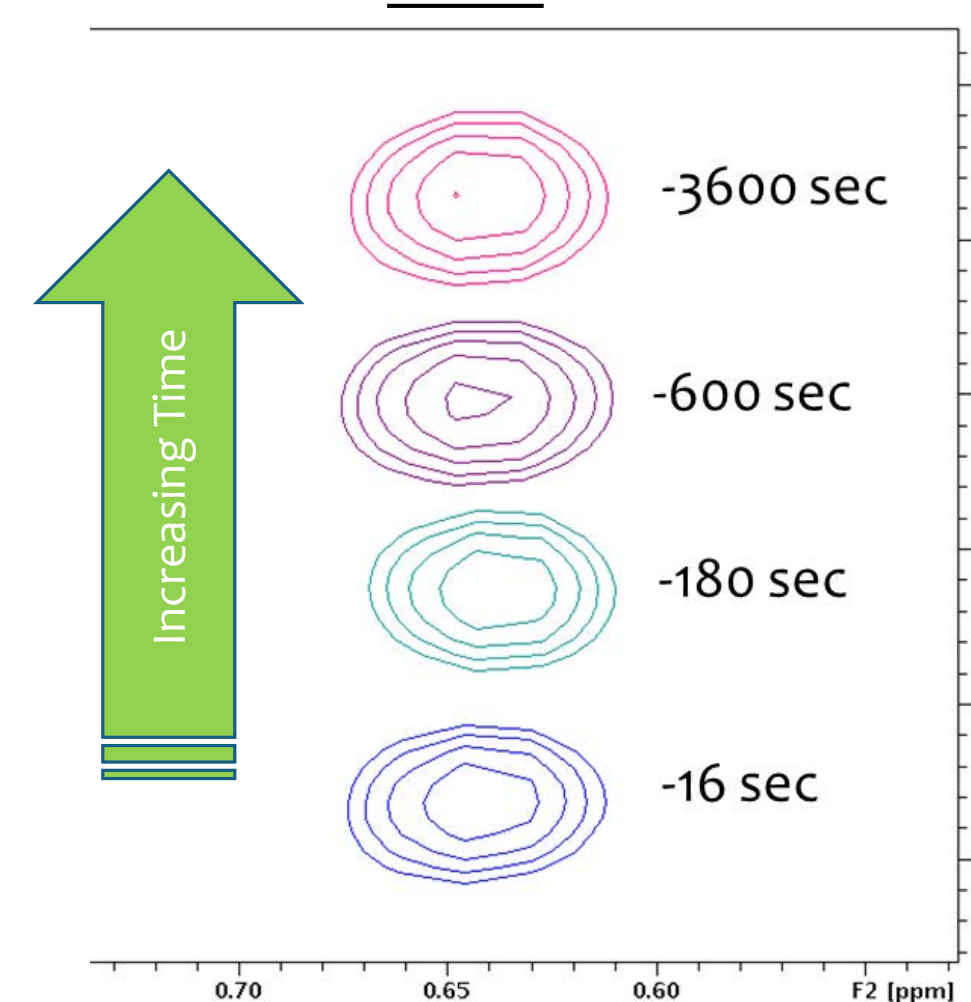
1D Proton Spectrum of Tobacco Plant



Time Buildup of Metabolites



DSS



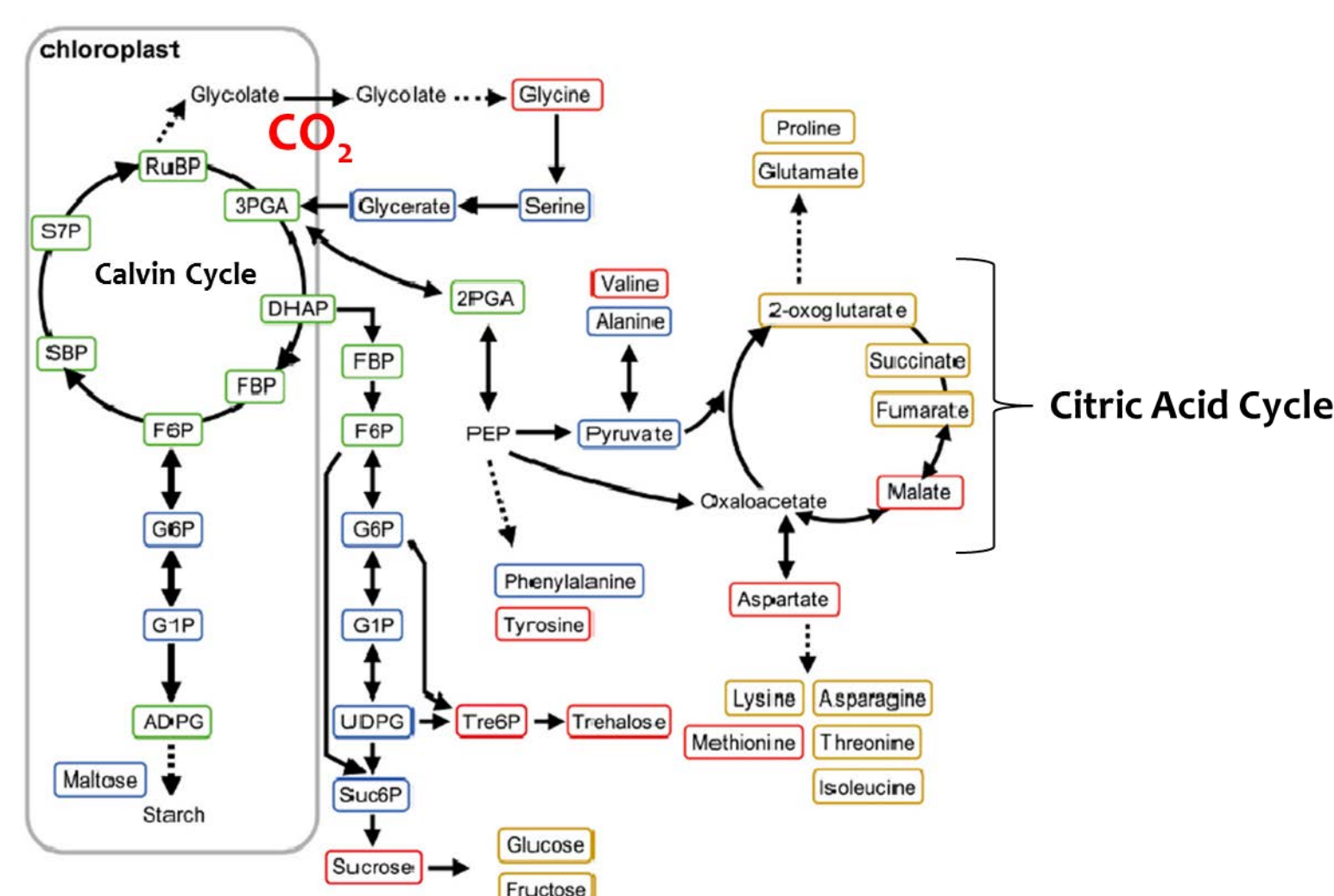
Based on the chemical reference (lower left) used for our NMR analyses (DSS), the scaling of each time plot was adjusted to ensure consistency between each time sample when comparing possible changes of metabolite concentrations. The data were collected with 1D proton analysis as well as 2D HSQC (Heteronuclear Single Quantum Coherence) on Bruker 500 MHz and 900 MHz NMR magnets.

Conclusions

- Based on my work, NMR offers a great way to monitor the metabolic system of the tobacco plant
- The spectrum found when analyzing the plant can serve as a great way to see what metabolites are changing and how
- In order to better understand the kinetics of the plant, longer labeling with carbon-13 should be accomplished in order to gain confident results (1 hr, 2 hr, 3 hr etc.)

NMR Role in Biofuel Production

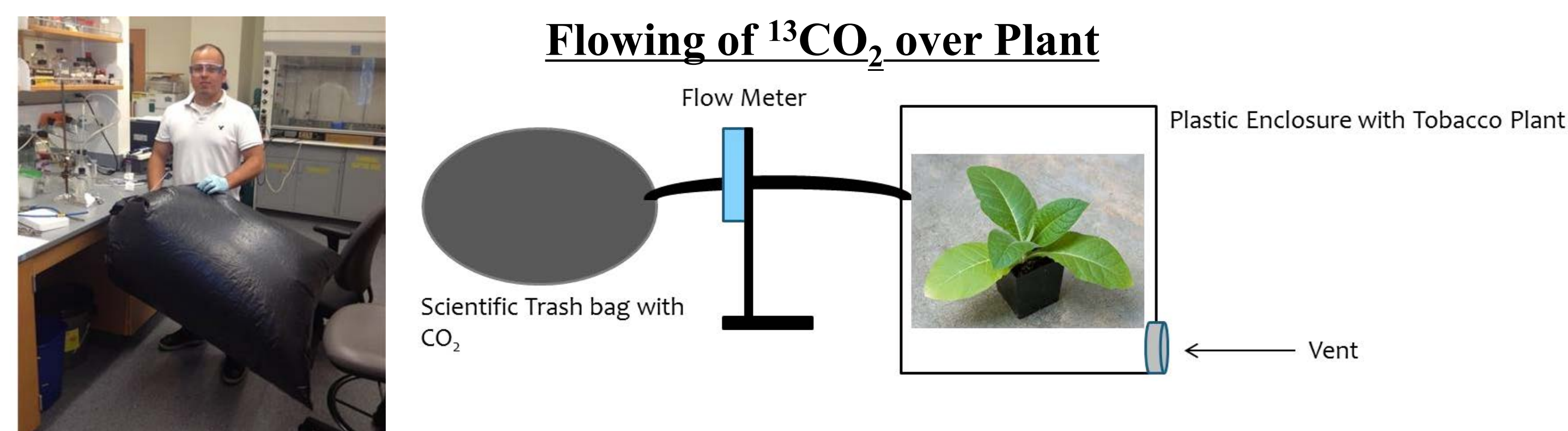
Plant Metabolic System



- Jansson Group of Lawrence Berkeley National Labs conducts genetic manipulation of plants
- Geneticists need a way to monitor metabolic changes after genes are modified in the tobacco plant
- NMR Spectroscopy gives us a way to monitor changes
- With the use of labeled carbon-13 CO₂ metabolic pathways can be monitored to see how long it takes to make particular metabolites
- If there are genetic changes that affect the plant in an adverse way, these results can give researchers a general baseline as to where a bottle neck could have occurred in the plants metabolism

Labeling Tobacco With ¹³CO₂

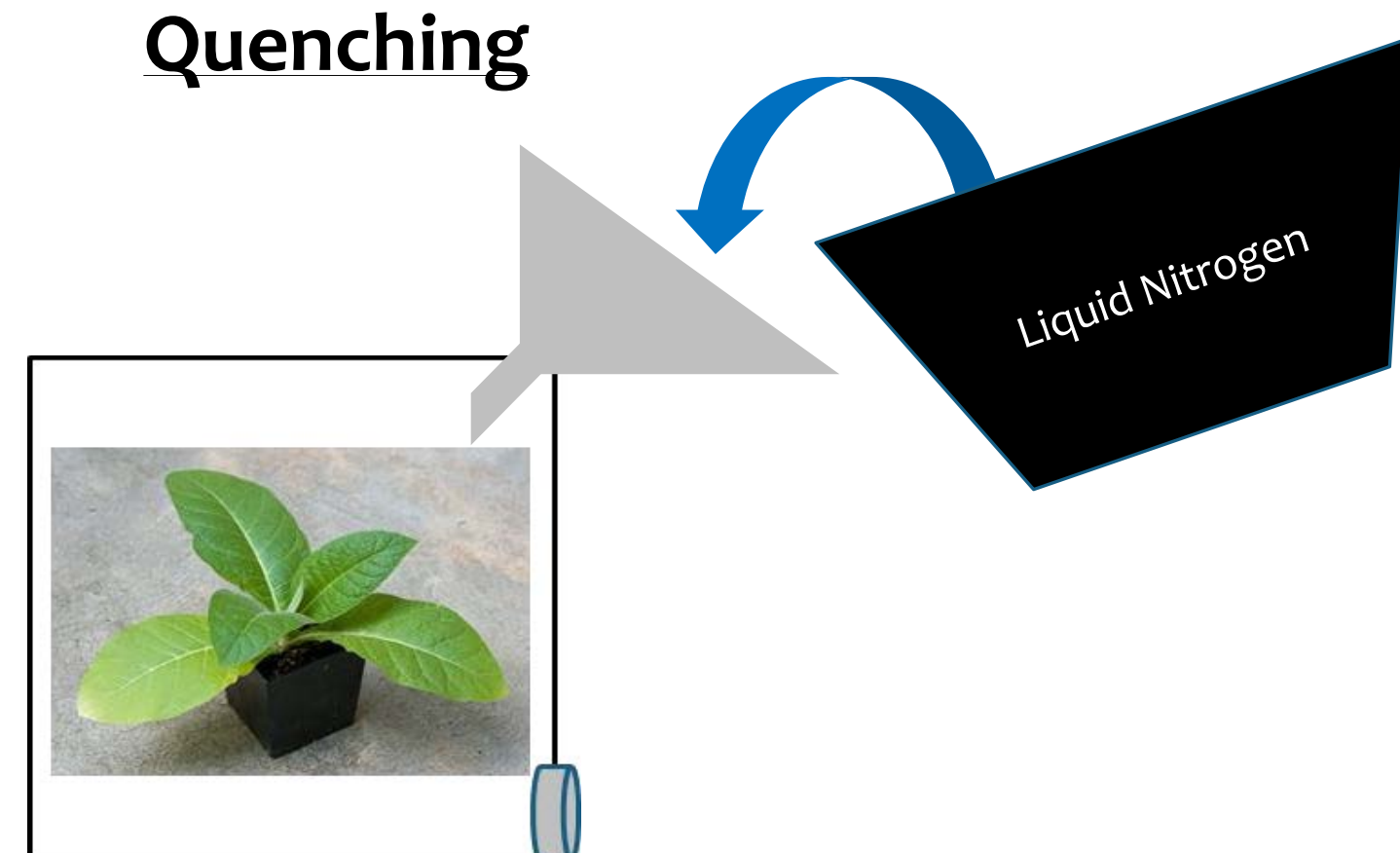
Flowing of ¹³CO₂ over Plant



- Flowing of labeled ¹³CO₂ done for 4 time periods
- 16 seconds
 - 180 seconds
 - 600 seconds
 - 3600 seconds

Quenching

Quenching is done so that the metabolism of the plant can be stopped at a precise time



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