in vivo Analysis of Plant Metabolites at Various Stages of its Life Cycle using TD-GC×GC-FID

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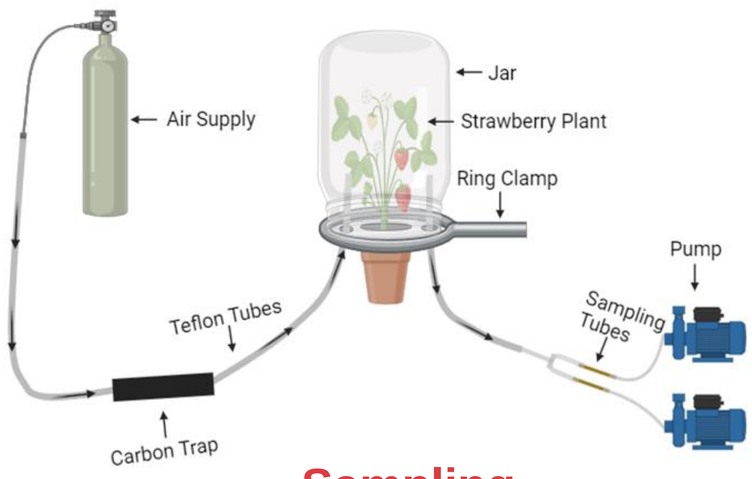
Introduction and Objectives

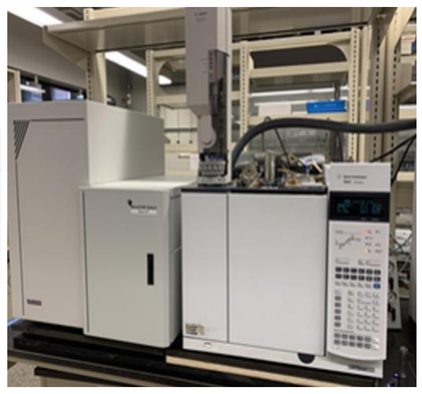
Plants produce many primary metabolites that aid in plant growth, development, and reproduction. Additionally, plants produce secondary metabolites that act to regulate plant defense, plant-to-plant communication, and attract pollinators. Many secondary metabolites get released from plants as volatile organic compounds (VOCs) that can be extracted and analyzed to understand how plants deter pests, attract pollinators, and communicate inter- and intra- species. Currently, there are several techniques to extract secondary plant metabolites but most methods involve irreversible damage to the plants. Therefore, we propose the use of an *in vivo* sampling system that enhances extraction of plant headspace volatiles without inducing any external interference[1]. In this study, two chili pepper and two strawberry plants were grown using a Click and Grow™ system with one of each plant type being grown in the dark and one in the light. They were then sampled using our *in vivo* plant sampling system and analyzed using thermal desorption comprehensive two-dimensional gas chromatography flame ionization detector (TD-GC×GC-FID) to determine how their VOC profiles developed over their life stages.

Materials and Methods



Sampling

1 h Sampling Time
Air Intake: 300-350 mL/min
Sampling Flow Rate: 50 mL/min



TD-GC×GC Analysis

Tube Desorption: 25 min, 250 °C, 50 mL/min
GC×GC Run Time: 62 min
[1]D Column: Rtx-5Ms, 30 m×0.25 mm×0.25 µm
[2]D Column: Rtx-17Ms, 5 m×0.25 mm×0.25 µm

GC Image
Software for Multidimensional Chromatography

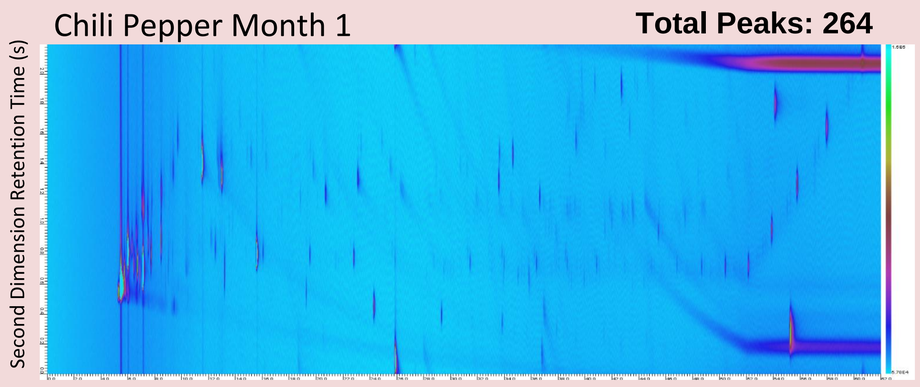
Data Processing

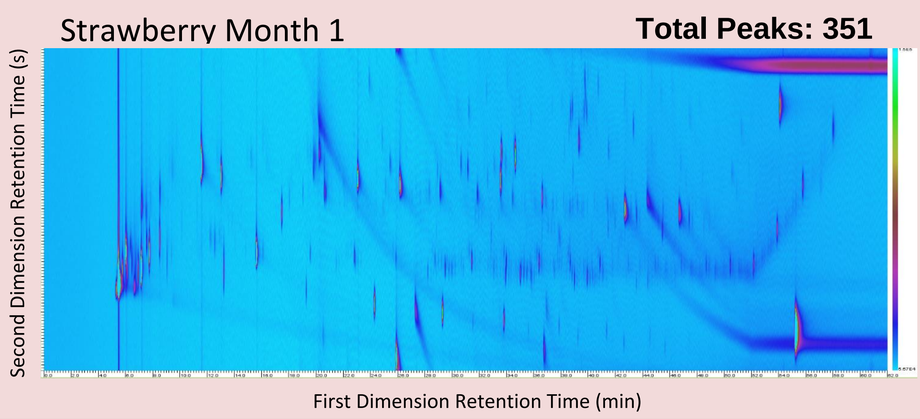
Data processed to assess the total peak area of the samples

Results

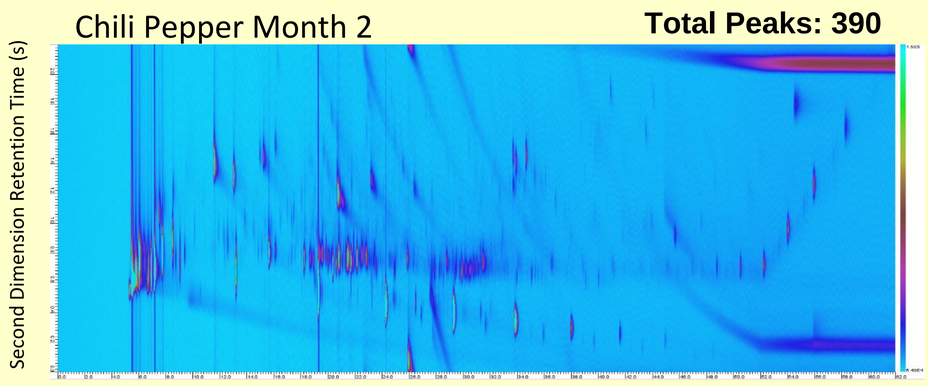
Previous Pilot Study

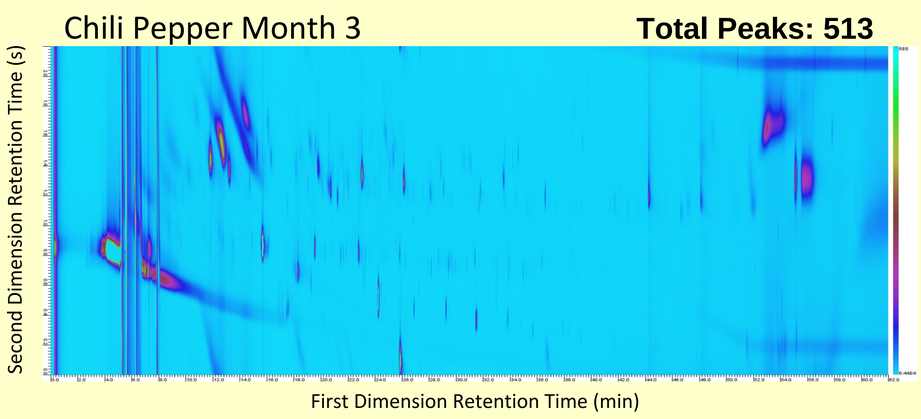
Analysis Between Species

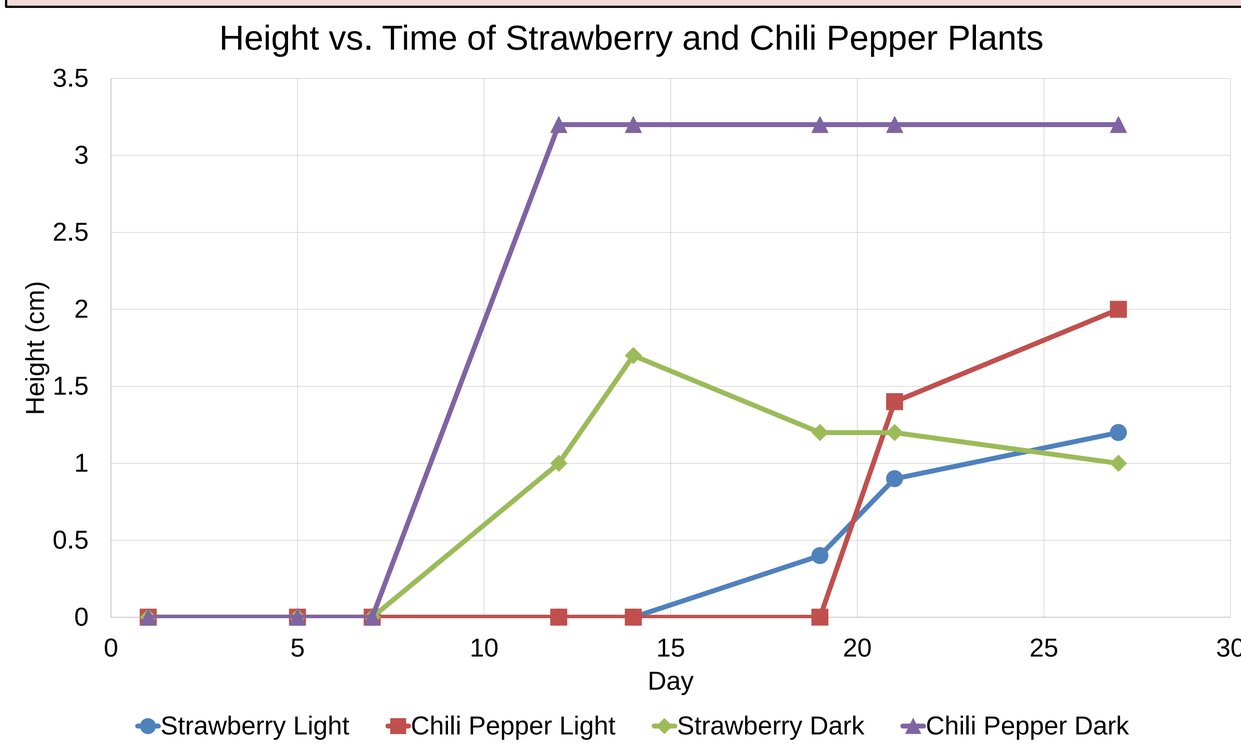


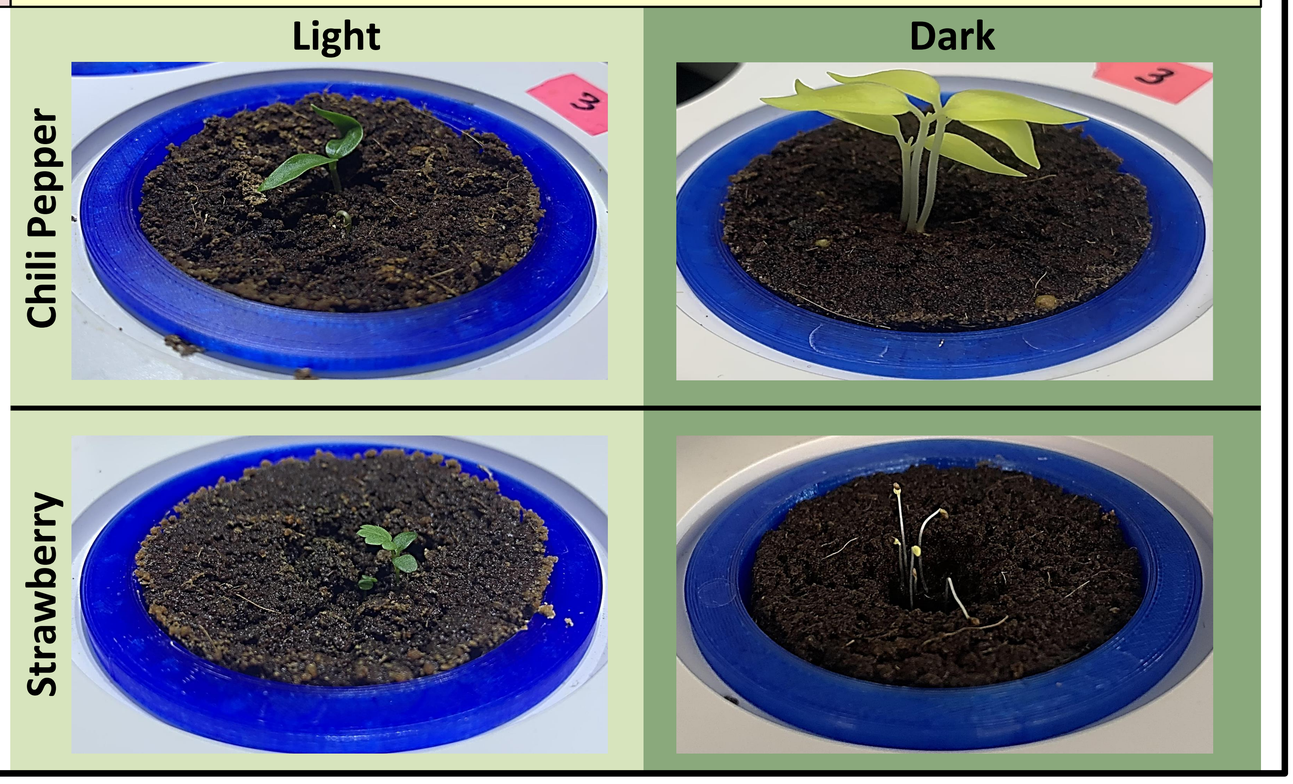


Analysis of Lifecycle









Conclusions

- We learned sampling every 2 days was excessive and time consuming. Sampling weekly or for every physical change would have been more effective
- In the future, changing the sampling time to a longer period would have been beneficial and could have allowed us to see more results
- Waiting longer for the sample to grow and having a smaller jar size might have allowed for better results as well
- Further work needs to be done on this project in order to obtain more accurate results using mass spectrometry which would allow us to determine what specific compounds were found in our results

Method figures created on BioRender.com

1- Schmidt, S. A.; Mesfin, E. Y.; De Silva Weeaddana, C.; de la Mata, A. P.; Costamagna, A.C.; Harynuk, J.J. "Analysis of in vivo plant volatiles using active sampling and TD-GC×GC-TOFMS". *Metabolites*. (Under review).

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