PHYTOTRON

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Phytotron Researcher Profile: Nick Sheppard

Describe your education and career path that has led you to your current research

My current research was inspired by some of the great courses and fantastic professors here at the University of Guelph. My main inspiration comes from the amazing Dr. Annette Nassuth. Taking Growth and Development when she was teaching it made me understand just how amazing plants could be, and I owe a lot to her for supervising me during my undergraduate research project.

Describe your research. What are your primary research questions?

My current research involves a novel approach to increasing seed yield from canola by manipulating starch synthesis and metabolism. By knocking out the endogenous Starch Branching Enzymes (SBE) in canola, and complementation via maize SBEs, we increased the total biomass of our canola plants. This was mostly due to an increase in stem thickness, and an increase in the number of branches (which consequently increases the number of seed pods, and therefore the seed yield). We are comparing our KO and transgenic lines to the unmodified wild-type cultivar to attempt to elucidate the mechanism behind the observed increase in yield/biomass. The primary objective is to quantify the changes in starch content throughout the plants, during the day and night, at multiple different stages of development.

Describe your work in the Phytotron. What more needs to be done to complete your current research project?

We use the Phytotron to grow a large number of canola plants, allowing us to have a sufficient number of replicates to establish trends in our different canola lines. This, in essence, requires 100 pots of canola, growing in controlled conditions for 2-3 months per experiment. To accomplish this, we require the large walk-in chambers and/or greenhouse space. The most critical part of these experiments is that we are able to control the light and dark periods, and can collect them at consistent times, and that these hundred pots can all be evenly watered via the automatic irrigation system. Additionally, I am looking at the root morphology of our canola lines, which requires the use of alternate soil media such as turface or vermiculite.

What is your favorite research tool or piece of scientific equipment that helps you carry out your research? Would your work be impossible without this equipment?

Outside of the growth chambers in the Phytotron, the most important piece of equipment I use is a spectrophotometer. Every single aspect of the canola plants that I am analyzing eventually needs to be measured in the spectrophotometer, so without one it would be a bit of a struggle to get many results.

If you had access to unlimited time, funding and equipment, where would you like to take your research? What questions would you like to tackle?

If that included copies of myself that I could spread the workload amongst, and unlimited space to grow plants, I would love to include more replicates and stages of development in my analysis. A greater number of replicates could give a better understanding of the range of starch levels (which can greatly vary from plant to plant) in each canola line. And more stages of development could give us a better idea of how starch is stored and used up in each line, as well as how this might lead to an increase in total plant biomass.

What is your favorite plant?

If saying canola is too on the nose, then I'd have to go with Tiger Lilies. My parents grow them in their garden, and they always bloom around my birthday, which has always felt like a really nice gift from nature.

Share something unique about yourself that isn't related to your research.

Perhaps, not too surprising, but I enjoy hiking, mountain climbing, going through parks, and biking. Really, anything that gets me out in nature is going to be something that I enjoy. I really love exploring and seeing how trails/parks are organized. Where do the paths go, what can you see along the way, and in the case of places like the Arboretum, where are the gardens, what is in them, and why?

Nick Sheppard is a graduate student working in the Emes/ Tetlow lab in the Department of Molecular and Cellular Biology.

Growth Chamber Setup for Sub-Ambient CO_2 Control

Introduction

A majority of the growth chambers in the Phytotron have been equipped with additive carbon dioxide (CO_2) control since their installation in the facility. CO_2 gas cylinders are centrally located, and CO_2 is delivered to each chamber by means of ½" copper pipe run throughout the facility and then reduced to ¼" nylon tubing for delivery into the air stream inside each chamber. CO_2 measurements are provided by the Vaisala GMP222 with a 0 – 3000 parts per million (ppm) range. Control of CO_2 injection into the chamber is by means of a solenoid valve activated by the chamber control system.

Additive CO_2 control serves two purposes for experiments being conducted in the chambers: 1) to maintain ambient CO_2 levels (~400 ppm) thus preventing CO_2 depletion during the lighting period, especially for plants like corn and rice; 2) for elevated CO_2 experiments where researchers want to saturate the air in the chamber with CO_2. For a detailed discussion on the importance of CO_2 in growth chamber experiments, see Chapter 4 of the Growth Chamber Handbook[1].

While at present there is concern regarding increasing levels of atmospheric CO_2, CO_2 concentrations have been below 270 ppm for the millennia before the Industrial Revolution, and were as low as 180 ppm by the end of the Last Glacial Maximum, about 20,000

years ago[2]. Despite the prevalence of low CO_2 conditions over the recent evolutionary history of plants, studies investigating plant responses to low atmospheric CO_2 concentrations are fewer than those examining the effects of future higher CO_2 levels.

In late 2019, we received a request from Dr. Andre Duarte to set up experiments in two chambers with sub-ambient CO_2 conditions. Dr. Duarte's work is focused on re-creating CO_2 and temperature conditions that happened in the recent geological past in order to investigate whether the interaction between plants and mycorrhizal fungi functioned similarly in the past compared to what is currently observed.

In the past, CO_2 depletion in chambers was accomplished by planting C_4 plants together with the C_3 plants being manipulated, relying on the C_4 photosynthesis to fix excess CO_2[3]. But this method required more chamber space and resulted in poor control of the CO_2 levels. Therefore, we need a system that can be relatively easily attached to modern CO_2-controlling chambers.

To begin planning for Dr. Duarte's experiment, I initially consulted the paper "Carbon Dioxide Within Controlled Environments; The Commonly Neglected Variable" by Mark Romer of the McGill Phytotron[4].

System Design – Part 1

The experiments were run in Conviron PGC20 chambers. My initial design simply took chamber air from the exhaust stack and directed it though a box filled with soda lime, then returned the scrubbed air to the chamber inlet. All of this was achieved using various pieces of residential furnace ducting material sized to fit the various parts of the chamber and a plastic storage container to hold the soda lime pellets (Figure 1 and 1a).

Design Flaws and Fixes

The initial design worked as shown by the early data from the chamber (Figure 2); but, it had several flaws.

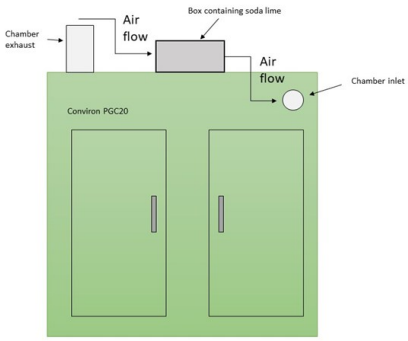

Figure 1: Original CO_2 scrubber design with air flow moving horizontally through the box of soda lime and directly to chamber air inlet.

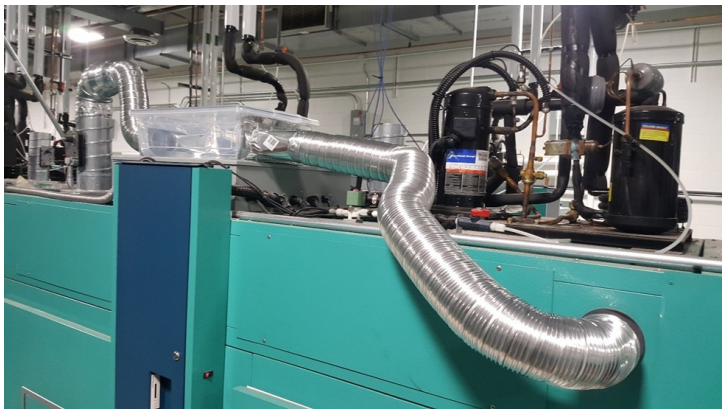

Figure 1a: Original CO_2 scrubber design with air moving horizontally through the box of soda lime and connected directly to the chamber inlet.

First I discovered that either the passive air flow between the exhaust and the inlet, was not quite strong enough to draw enough scrubbed air into the chamber or the chamber was excessively leaky and ambient level CO_2 air from the room was being drawn into the chamber at a higher rate than scrubbed air. To address the potential for leaks, I made sure that all duct joints were well taped with foil tape, that both doors closed tightly, and the door seals were not damaged. To address the air inlet issue, I decided to move the return air tube directly in front of one of the circulating fans in the chamber (Figure 3). This new placement ensured that air was being pulled through the scrubber system by the fan.

The increased airflow led to the second problem I discovered in the box of soda lime. Once the soda lime

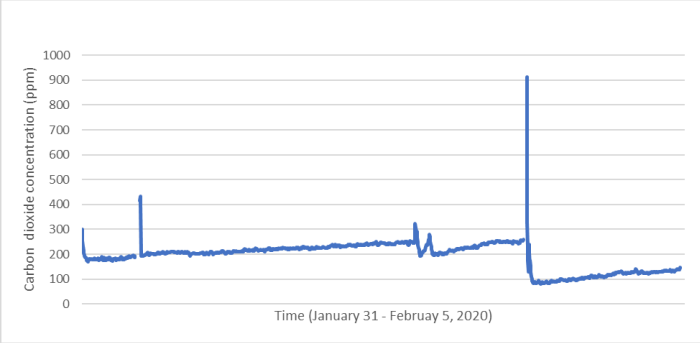

Figure 2: CO_2 concentration over time using the original scrubber design. Note the over compensation of the scrubber system after the large spike, likely caused by opening the chamber door for watering.

Figure 3: The location of the scrubbed air return was moved in front of one of the chamber's circulating fans in order to increase the flow of air through the soda lime box.

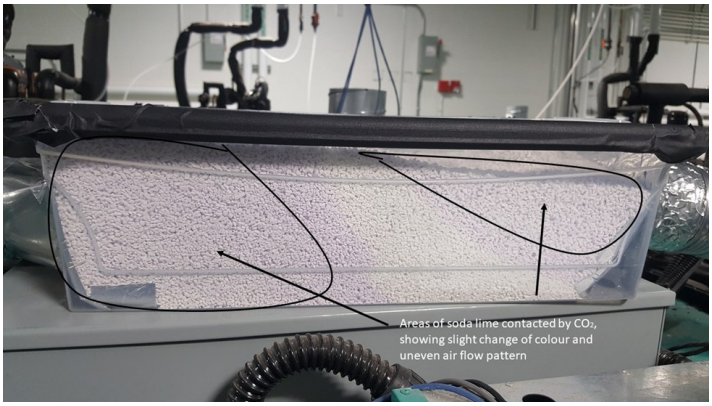

Figure 4: The original design caused uneven airflow through the soda lime. Areas of spent soda lime are outlined as the colour contrast is difficult to detect in the photo.

started to absorb CO_2 and change colour from white to purple, I was able to observe a clear and very uneven pattern of usage (Figure 4).

System Design – Part 2

To correct the above-mentioned issues, I set out to redesign the soda lime container that would allow for more even air flow through the bed of material. I decided to try directing the air upwards through the soda lime. Figures 5 and 5a show a diagram and picture of the redesigned soda lime box. The air flows up through the bed of soda lime and is returned to the chamber by ducting at the top of the box (Figure 6 and 6a). This design uses a piece of perforated steel to diffuse the air and allows it to flow much more evenly through the bed of soda lime. Further modifications can be made by restricting air directly over the duct work and forcing it to diffuse to the far corners of the box. Finally, I also placed foam tape along the edge of the soda lime box to ensure the lid fit tightly, preventing any air leaks. As an extra precaution, the lid is taped tightly on to the box.

The second major change in the new design was to make use of the damper motor on the chamber exhaust (Figure 7). In high CO_2 settings, this damper functions to preserve high CO_2 concentrations in the chamber by staying closed when settings are above 800ppm and only opening to exhaust chamber air if the readings go over the CO_2 set point.

In the low CO_2 experiment, I wanted to use the damper motor to control the amount of air flowing through the soda lime, thus preventing the chamber from having to continually inject CO_2 to maintain the 180ppm set point. The damper motor should stay closed, cutting off airflow to the soda lime as long as the CO_2 level is at or below the 180ppm set point. The damper motor should only open if the CO_2 readings rise above 180ppm, thus allowing chamber air to flow through the soda lime and return elevated CO_2 levels to the set

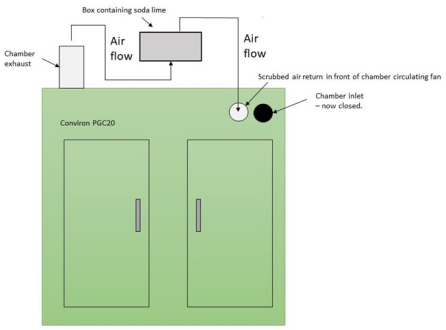

Figure 5: Diagram of redesigned CO_2 scrubber design with new airflow pattern

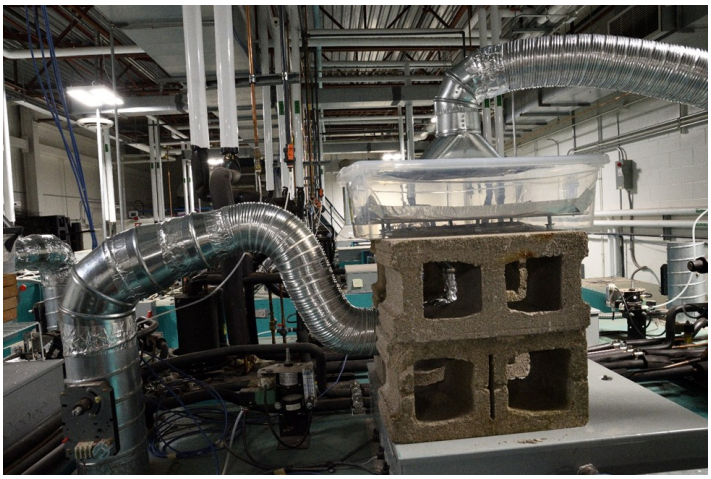

Figure 5a: Redesigned scrubber system showing chamber air exhausted up through the box of soda lime and out the top before it gets returned to the chamber growing area.

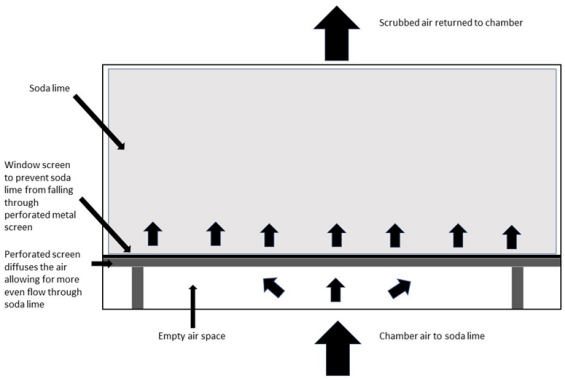

Figure 6: Detailed diagram of redesigned soda lime box with air entering from below, showing diffused and even air flow pattern through soda lime.

point. The exact opposite of how the damper is set up to work!

In order to get it to work, I forced the damper to stay closed at all times (a -1 setting in Conviron's program scheme). Then I set up a non-critical alarm limit for a high CO_2 reading of 185ppm. With this alarm limit, the chamber 'thinks' that the CO_2 is at a dangerous level and overrides the forced closed (-1) setting and opens the damper, allowing chamber air to flow through the soda lime. By setting the alarm as non-critical, the chamber does not shutdown or activate our alarm auto-dialer.

Conclusion

With the improved design, we are able to maintain 180 ppm for several weeks at a time (Figure 8). When the soda lime becomes exhausted, we initially start to see rising levels during the overnight period (Figure 9). This is a sign to replenish the soda lime! Thanks to Mark Romer for taking the time to speak with me about this project and to Dr. Andre Duarte for his patience as I worked through the different designs!

References

[1] Growth Chamber Handbook. **Edited by R.W. Langhans and T.W. Tibbitts, North Central Regional Research Publication No. 340, Iowa Agriculture and Home Economics Experiment Station Special Report No. 99.** (Available electronically: https://www.controlledenvironments.org/growth-chamber-handbook/)

[2] Petit, R.J., Raynaud, D., Basile, I., Chappellaz, J., Ritz, C., Delmotte, M., Legrand, M., Lorius, C., Pe, L., 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399, 429–413.

[3]Sharma RK, Griffing B, Scholl RL. 1979. Variations among races of *Arabidopsis thaliana* (L.) Heynh for survival in limited carbon dioxide. Theoretical and Applied Genetics 54: 11–15.

[4] Romer, M. 2001. Carbon dioxide within controlled environments; The commonly neglected variable. Proceedings of the International Conference: Controlled Environments In The New Millennium. (Available electronically: https://www.mcgill.ca/phytotron/files/phytotron/co2_controlled_environments.pdf)

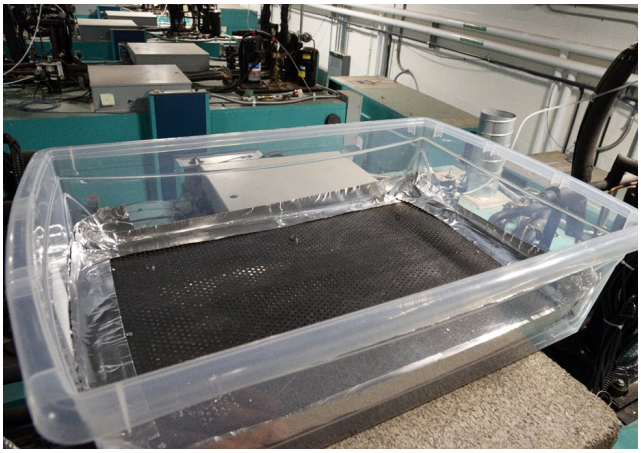

Figure 6a: Interior of the soda lime box showing the black window screen layer, with edges taped to prevent soda lime pellets from falling through to the empty air space.

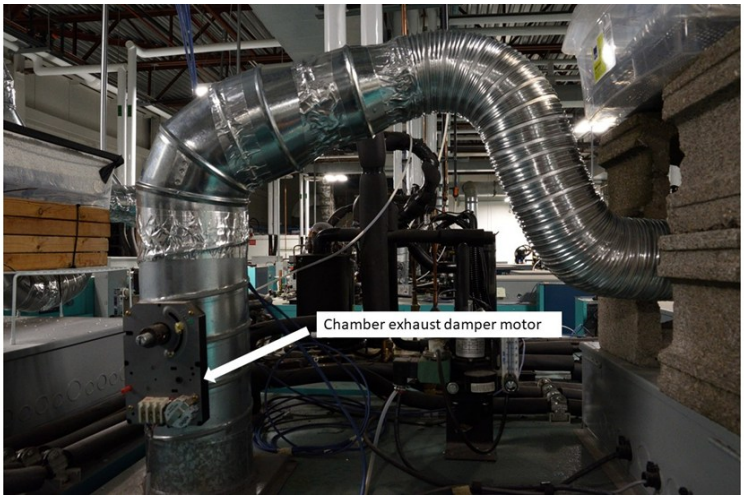

Figure 7: The chamber exhaust damper motor used to control the flow of air through the soda lime. If the chamber CO_2 remains at or below the 180 ppm set point, the damper remains closed, restricting air flow through the soda lime. If the chamber CO_2 climbs above the 180 ppm set point, the damper opens, allowing chamber air to flow through the soda lime to scrub excess CO_2.

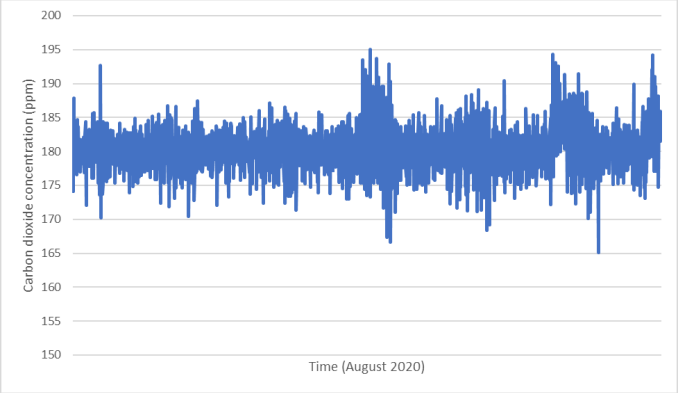

Figure 8: CO_2 concentration August 2020.

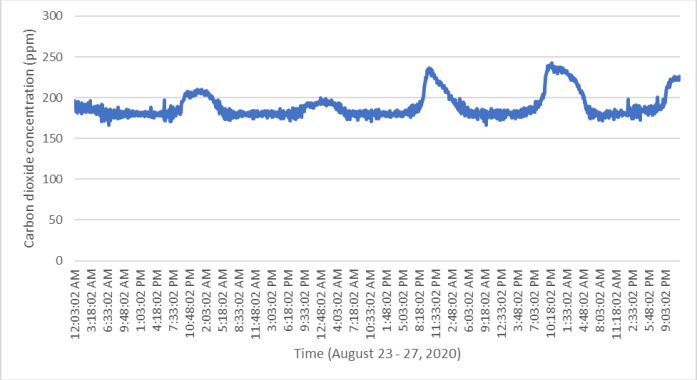

Figure 9: CO_2 concentration over time for 4 days in August 2020. Note the increase in CO_2 levels during the night periods – a sign that it is almost time to change the soda lime.

This article, written in collaboration with Dr. Andre Duarte, was originally published in the Association of Education and Research Greenhouse Curators Newsletter, Winter 2021, Volume 32, Number 3.

At the time, Andre was a post-doc in the Maherali lab.

Phytotron Plant Sale

When: Tuesday May 12th, 10am – 3pm

Where: Science Complex, outside near the doors of the Waasamowin (Atrium)

A wide variety of plants for your garden! Tomatoes, peppers, herbs, annuals!
Limited number of Phytotron T-shirts!

***Electronic Payments ONLY, no cash sales!

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