PLANT STEM UNDERGRADUATE SYMPOSIUM

July 20th, 11:00am -1:00pm

ENGR 3rd floor student study area

Richard and Moonyeen Anderson Engineering building

Biological Engineering REU 2023

Utah State University

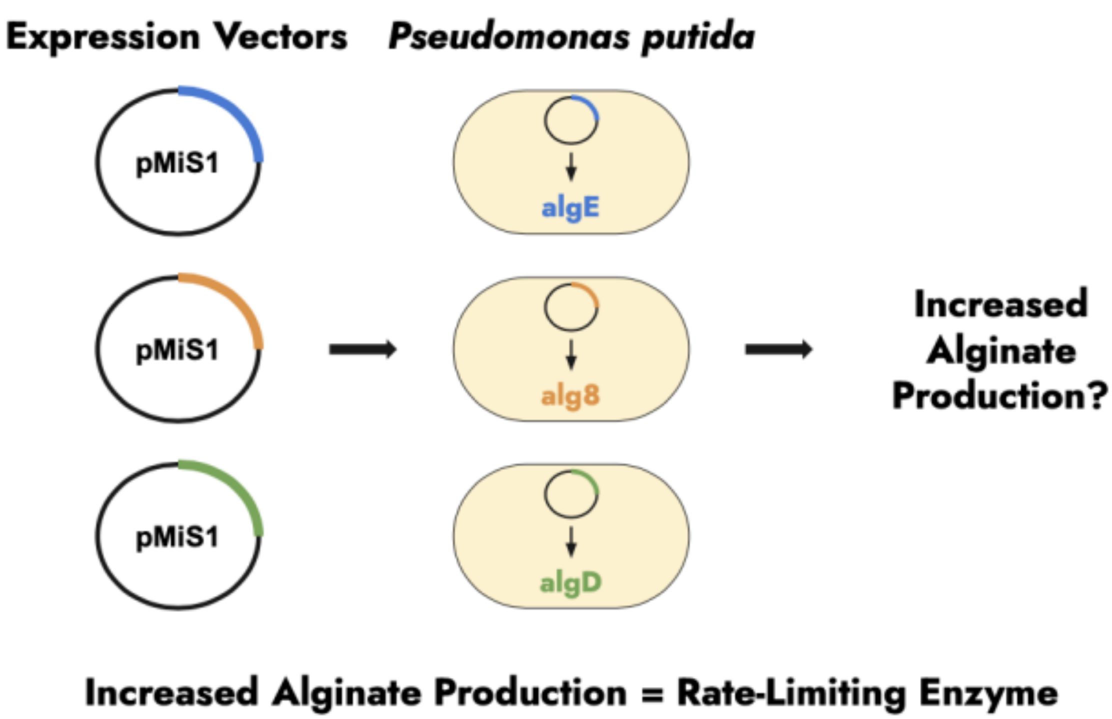
Engineering Pseudomonas putida for Increased Alginate Production

Virginia Akins[a] *, Lindsey Clark[b] *, Jixun Zhan[c]

(a) University of Missouri, (b) Illinois Institute of Technology, (c) Department of Biological Engineering, Utah State University

Alginate is an extracellular polymeric substance produced by various soil microbes including pseudomonas putida. This polysaccharide is used in the agricultural, textile, and food industries as a coating and thickening agent. While the majority of alginate is sourced from brown seaweed, the extraction process is expensive and water intensive. Engineering P. putida to produce more alginate could introduce other practical sources of this compound.

Overexpressing the rate limiting enzyme can increase the production of alginate. This protein can be identified by overexpressing individual genes in the alg gene cluster and then quantifying the alginate produced using the carbazole assay. By cloning this gene and then ligating it into an expression vector, higher levels of alginate are expected. More sources of alginate can be discovered by further studying bacteria species and quantifying any alginate they produce.

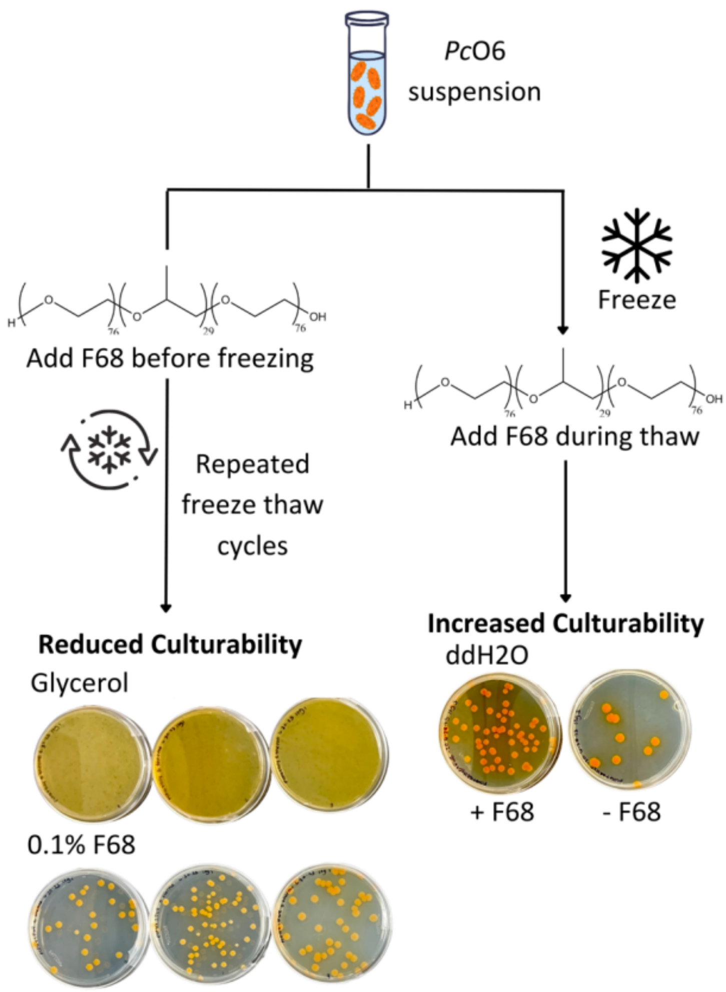


Response of a Pseudomonad to Cryogenic Stress & the Role of Pluronic F68 as a Cryoprotectant

Amanda Streeter[1]; David Britt[2]; Anne Anderson[3]

[1] Department of Biological Sciences and Department of Chemistry, State University of New York at Oswego, [2] Department of Biological Engineering, Utah State University, [3] Department of Biology, Utah State University

Understanding the response of *Pseudomonas chlororaphis* O6, PcO6, to cryogenic stress is relevant to how this plant probiotic can survive winter subzero soil conditions; freeze-thaw stress also is a problem for long-term laboratory storage. This work investigated whether Pluronic F68 would mitigate the negative effects of freeze-thaw cycles on the bacterium, while also addressing during which phase of the freeze-thaw cycle the addition of F68 would be the most beneficial. The study compared the culturability of the bacterium after repeated freeze-thaw cycles in a -20° C freezer when stored in sterile water, 15% glycerol, 0.1% F68 or 1% F68. The culturability of the bacterium when F68 was added during the thawing phase of the freeze-thaw cycle was also examined. No protection was seen with storage in F68, but the addition of F68 during thawing had a positive effect on culturability.

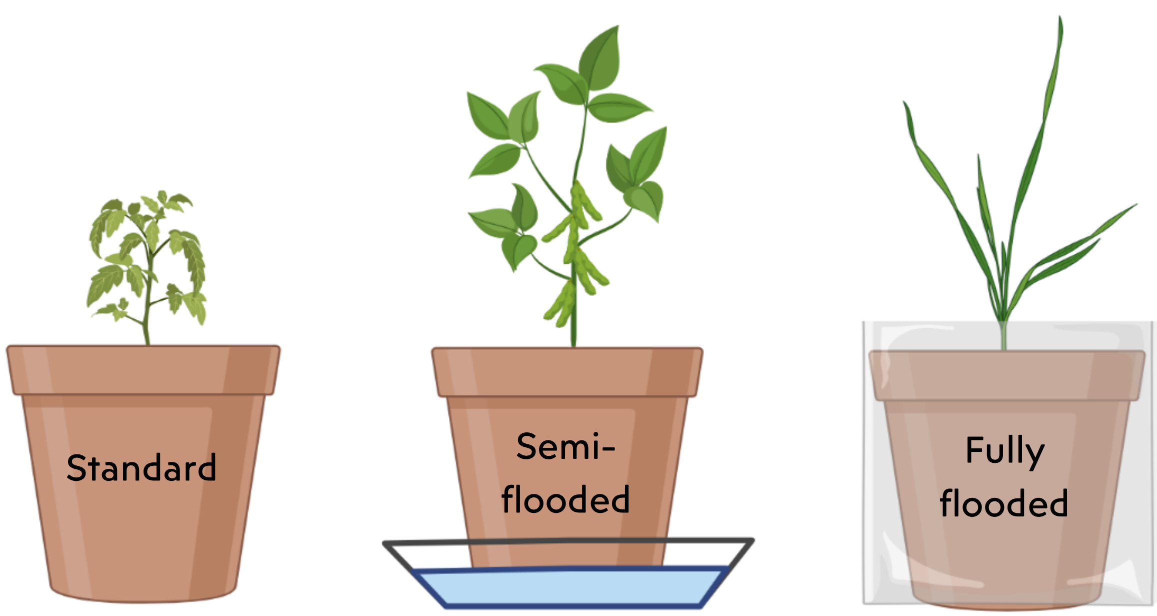


Tolerance to limited soil oxygen in crop plants

Victoria Nicholes[1, 2], Noah Langenfeld[2], Bruce Bugbee[2]

[1] Plant Ecology Lab, Shepherd University, WV, USA, [2] Crop Physiology Lab, Utah State University, UT, USA

Plants are grown in closed systems with specific water parameters in space. Crop species have different optimal moisture contents to optimize growth and ensure survival when grown in space environments. We grew five crop species in three different experimental groups to investigate the optimal moisture content. Our standard experimental group was given nutrient solution by pot weight or drip irrigation lines, the semi-flooded group was placed in a tray filled with nutrient solution, and the fully flooded group was kept saturated with nutrient solution in bagged pots to restrict drainage. Peas were the least tolerant to low soil oxygen as the fully flooded experimental group stopped growing within twelve days. Hoyt soybean was the most tolerant to low soil oxygen. However, three of our five species had the highest biomass index when grown in semi-flooded conditions. Dry biomass harvest increased by 77.6% for peas, 161.5% for soybeans, and 67.6% for wheat grown in the semi-flooded treatment compared to the standard. Pepper and tomato dry biomass decreased by 57.1% and 40.9% respectively in the semi-flooded treatment compared to the standard. Soybean was found to be the most tolerant species to low soil oxygen, followed by wheat, tomato, pepper, and pea.



Could a Naturally Occurring Iron Phosphate Mineral Be an Effective Alternative to Chelate Based Iron Amendments?

Eric DuPont Jr[a,c], Jenna Hughes[b,c], Li-Ting Yen[c,d], Astrid Jacobson[c]

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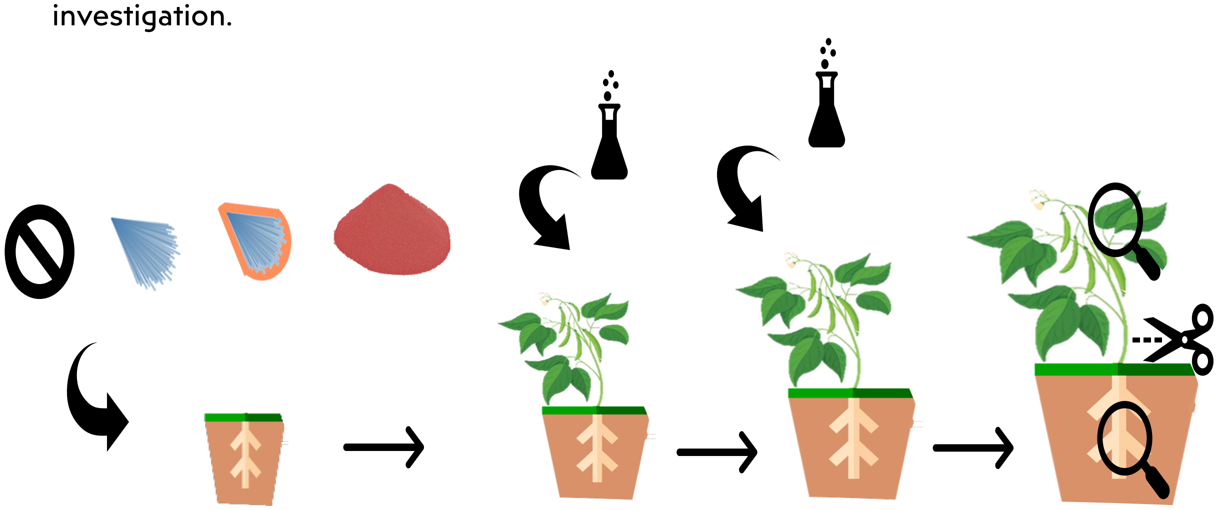
Iron (Fe) is an essential plant micronutrient. Fe deficiency leads to chlorosis, decreased crop yields and quality. In high pH calcareous soils, Fe is unavailable for plant uptake. To overcome this challenge, soils are typically amended with an Fe chelate (Fe-EDDHA). Fe-EDDHA is costly to produce and leaches readily from the rooting zone due to its solubility in water. We are investigating metavivianite, a naturally occurring iron phosphate mineral that can be recovered from wastewater, as a bioavailable Fe crop amendment with low leaching potential. We also investigated coating the metavivianite particles with chitosan, a polymeric coating that gives the particles a positive charge, thus enhancing their ability to be held by negatively charged soil surfaces. Beans grown with metaVT and CT-metaVT will provide yields which are not significantly different from plants fertilized with Fe-EDDHA. Four treatments (double distilled water -- control, metavivianite nanoparticles, chitosan-coated metavivianite nanoparticles, and Fe-EDDHA – positive control) were prepared in triplicate. Fe amendments were applied at a rate of 5mg Fe/ Kg soil. Beans (*Phaseolus vulgaris*) were grown for 50 days in Millville series soil in an environmental growth chamber set to a 16:8 light cycle and watered to field capacity daily. Half-strength Hoagland's solution modified to lack Fe was applied to each plant on days 22 and 37. Our data show that metavivianite may be a suitable alternative amendment to Fe-EDDHA. The proliferation of flowers on the metaVT and CT-metaVT treatments suggests that metavivianite provides superior benefit relative to Fe-EDDHA, thus warranting further investigation.

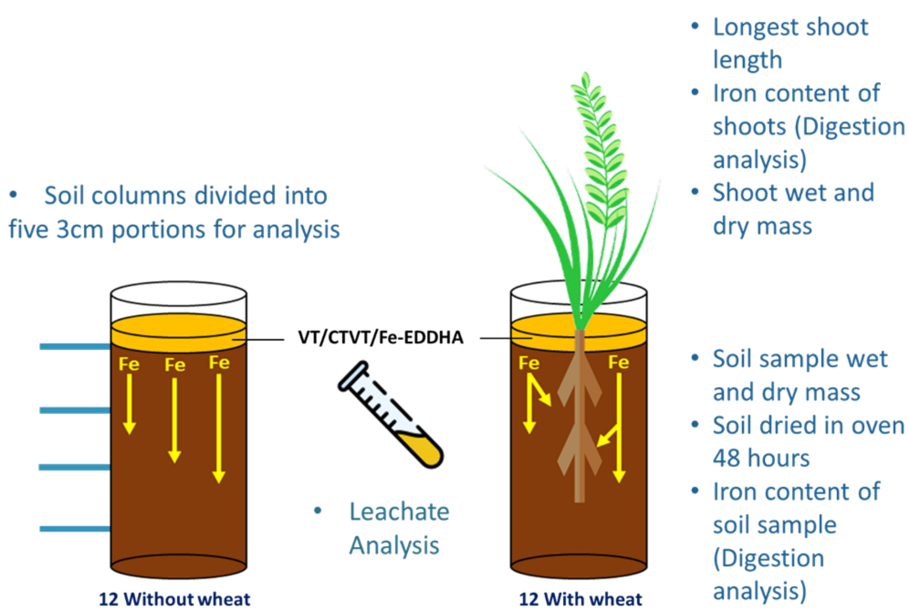


Metavivianite for Sustainable Plant Growth: Fe Mobility and Leaching Through Soil

Jenna Hughes[1], Eric DuPont Jr[2], Li-Ting Yen[3,4], Astrid Jacobson[3]

[1]Department of Civil, Environmental, and Construction Engineering, University of Central Florida, Orlando, FL, USA, [2]Massachusetts College of Liberal Arts, North Adams, MA, US, [3]Department of Plants, Soils, & Climate, Utah State University, Logan, UT, USA, [4]Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan

Iron (Fe) is a plant essential micronutrient. In calcareous soils Fe is not bioavailable and needs to be supplemented in a form that plants can take up. The common iron amendment, Fe-EDDHA is expensive and leaches from the rooting zone. Vivianite is an iron phosphate mineral that can be precipitated from wastewater to provide bioavailable iron to plants. Metavivianite (metaVT) is a partially oxidized form of vivianite. Chitosan (CT) is a naturally-derived biopolymer that can be used as a coating to improve sorption to soil surfaces. This study examines Fe leaching from the rooting zone of wheat (Triticum aestivum) grown in calcareous soil (Millville series) columns. We compared metaVT and CT-metaVT to the iron chelate, Fe-EDDHA, at 5 mg Fe/kg soil. The amendments were incorporated into the top 10 g soil of 320 g (total mass) soil columns planted with winter wheat or left fallow, and watered daily. Leachates were collected from d10-d15 and analyzed for total Fe. On d15 wheat from the columns was harvested, shoot length and wet and dry masses determined. The soil was extruded from the plastic sleeves and divided into five layers. Soil from each layer was analyzed for total and plant-available (DTPA extractable) iron. Results indicate that wheat grown with metaVT and CT-metaVT perform as well as the Fe-EDDHA amendment and significantly better than the control. Very little Fe leached from metaVT and CT-metaVT amendments relative to Fe-EDDHA. This study suggests that metaVT and CT-metaVT are viable alternatives to Fe-EDDHA as Fe crop amendments.

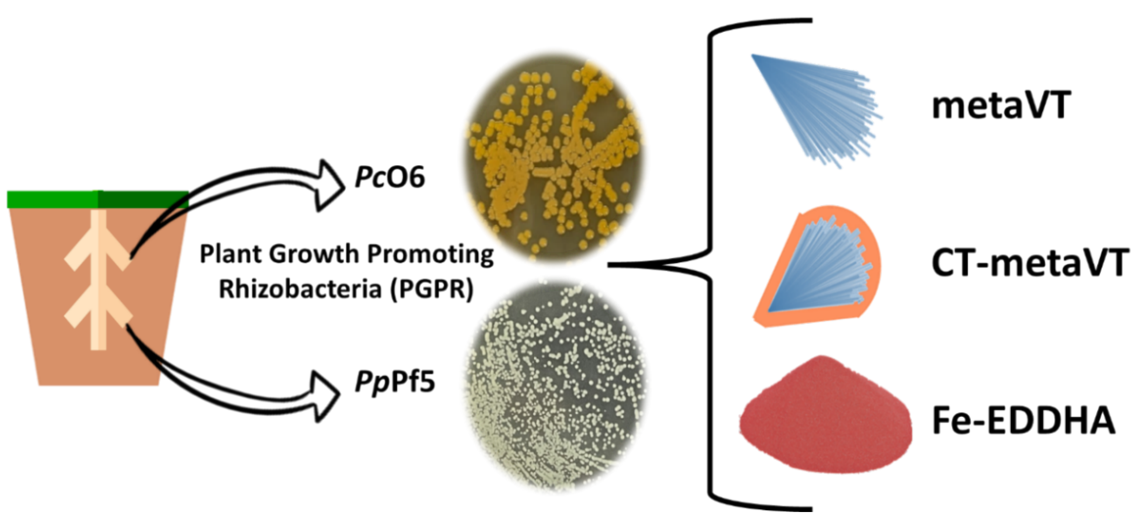


Metavivianite for Sustainable Plant Growth: Promotion of Fe-Dependent PGPR

Jenna Hughes[1], Eric DuPont Jr[2], Li-Ting Yen[3,4], Astrid Jacobson[3]

[1]Department of Civil, Environmental, and Construction Engineering, University of Central Florida, Orlando, FL, USA, [2]Massachusetts College of Liberal Arts, North Adams, MA, US, [3]Department of Plants, Soils, & Climate, Utah State University, Logan, UT, USA, [4]Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan

Iron and phosphate bioavailability is highly limited in calcareous soils. metavivianite, a mineral that can be extracted from wastewater, (ferric-ferrous phosphate) is being suggested as possible agricultural amendments. Since soil bacteria as well as crops require Fe and P, in this simple culture study we aim determine how the growth of *Pseudomonas clororaphis* O6 and *Pseudomonas protegens* Pf5, beneficial soil bacteria that can be isolated from several crops including wheat and cotton, is affected by Fe and P from meta-vivianite. The Fe source will be compared with Fe-EDDHA (Ferric ethylenediamine-di-(o-hydroxphenylacetate)), a conventional synthetic chelate, is used as a Fe amendment. The bacteria were incubated in solutions of half-strength Hoagland solution (pH 8.4), modified to omit Fe and amended with the minerals of interest and a carbon source. The control consisted of complete, half-strength Hoagland solution with 1 g/L sucrose. The effect of salinity stress on the two soil bacteria treated with Fe and P amendments were investigated with EC-adjusted half-strength Hoagland solution (pH 8.4; EC 10 dS/m). After 24, 48, and 72 hrs, subsamples from each incubation bottle were serially diluted on Luria-Bertani (LB) plates (*Pc*O6) or Trypticase Soy Agar (TSA) plates (*Pp Pf*5), allowed to grow for 48 hrs, and colony forming units counted. Initial results show that *Pc*O6 growth was not affected by the Fe source. *Pp* Pf5 growth improved with Fe sources.

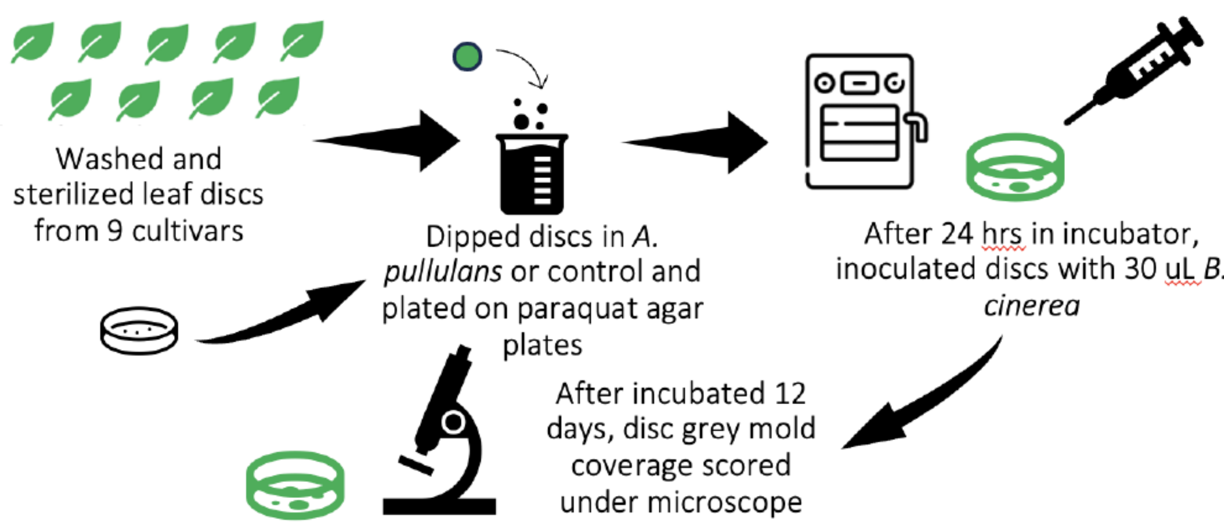


Is Microbe-Assisted Resistance to Grey Mold Cultivar-Dependent?

Helen Vaughan[1,2]*, Emily Burgess[2], Robert Schaeffer[2]

[1]Middlebury College, [2]Utah State University

Agriculture in Utah is dominated by pastureland and hay/alfalfa for cattle feed. For farmers looking to diversify their crops with an organic, high profit, alternative, high tunnel-grown strawberries are a perfect option. However, a major issue with strawberries grown in high tunnels is the fungus *Botrytis cinerea*, which causes grey mold. Grey mold is a major cause for fruit rejection post-harvest (Petrasch et al. 2019; Xiong et al. 2018). However, evidence suggests that the microbial biocontrol agent (mBCA) *Aureobasidium pullulans* may help prime plants' immune systems to fight off *B. cinerea*, lowering grey mold levels (Ippolito et. al 2000; Zeng et al. 2023). In this experiment, we tested how *A. pullulans* affected *B. cinerea* growth on 9 different strawberry cultivars using a leaf disc assay. We found that it was effective in lowering grey mold growth across cultivars, although cultivars had differing levels of response. In the future, we will use RT-qPCR methods to determine the levels of pathogenesis-related proteins (PR proteins) expressed across cultivars, focusing on beta-1, 3-glucanase and chitinase, which are involved in breaking down pathogen cell walls (Yamamoto et al. 2015; dos Santos et al. 2023). We will also continue to collect data on the marketability of strawberries produced across different cultivars, providing farmers with the best information to help them decide which strawberry cultivars to grow, what challenges to expect, and what solutions there are to those problems.

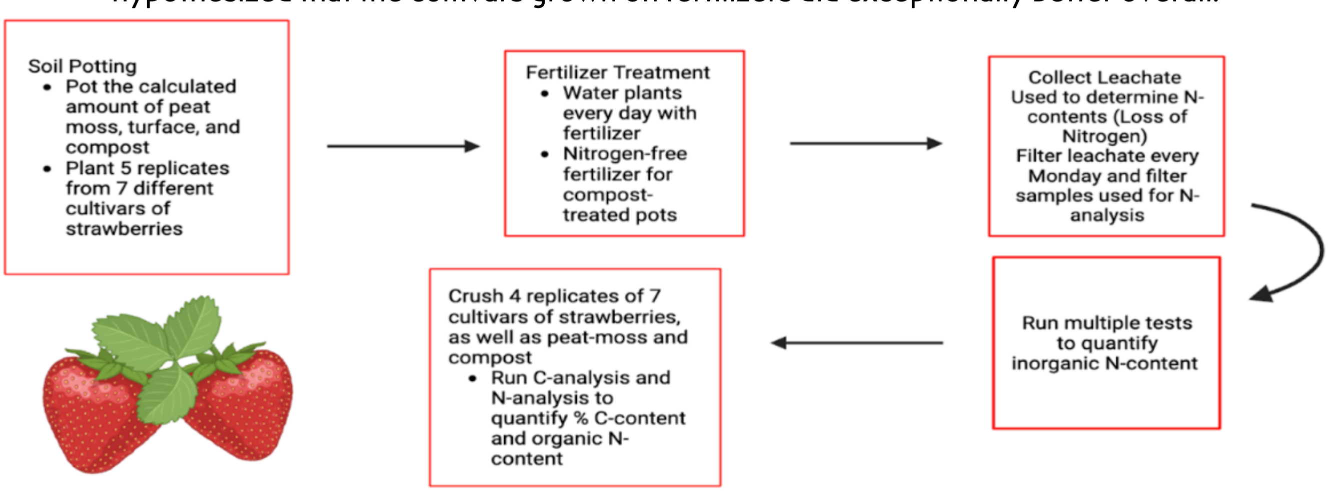


Strawberry Response to Compost Versus Inorganic Nitrogen Fertilization

Elizabeth De LaTorre[1], Kauai Paule[1], Jennifer Reeve[2]

Department of Crop Science, College of ACES, University of Illinois at Urbana–Champaign[1], Department of Natural Sciences, College of Life Sciences, University of Hawai'i at Mānoa[1], Department of Soil Science, College of Agriculture and Applied Science[2]

In this research project, we have compared the biomass, disease response to gray mold caused by botrytis, and nitrogen uptake vs. loss of compost-grown and fertilizer-treated strawberry plants from seven different cultivars. Organic cultivation strictly regulates allowable products for fertilization and pest control. Growers rely on organic nitrogen sources such as compost and other waste products as it enhances plant disease resistance by stimulating diverse microbial activity, suppressing harmful pathogens, and creating a healthier soil ecosystem. However, the use of compost comes with various challenges, such as variable nutrient content and difficulty predicting nitrogen availability. Strawberries vary in their ability to grow on organic sources of nitrogen. Cultivars that grow well on organic nitrogen sources such as compost and are disease resistant will provide improved cultivar recommendations for organic growers. Seven different strawberry cultivars were grown in pots with two treatments—commercial fertilizer vs. compost and nitrogen-free fertilizer. Leachate was collected daily from each pot, and a colorimetric assay was performed to determine the changes in loss of inorganic nitrogen concentrations (ammonium and nitrate) through leachate every week. After six weeks, the plants were assessed for leaf chlorophyll (SPAD) and divided into individual tissues to determine dry mass: crowns, leaves, and roots. Since the data has been collected, we will determine the differences in comparing compost versus inorganic nitrogen soil used to grow these seven different cultivars. Though some cultivars performed better on compost than fertilizer, as evident through the tissue mass and SPAD measurements, we hypothesized that the cultivars grown on fertilizers did exceptionally better overall.



Exploring the Effect of a Biocontrol on the Scent-Mediated Attraction of Bumblebees to Strawberry

Zoe Smutko[1], Emily Burgess[2], Robert Schaeffer[2]

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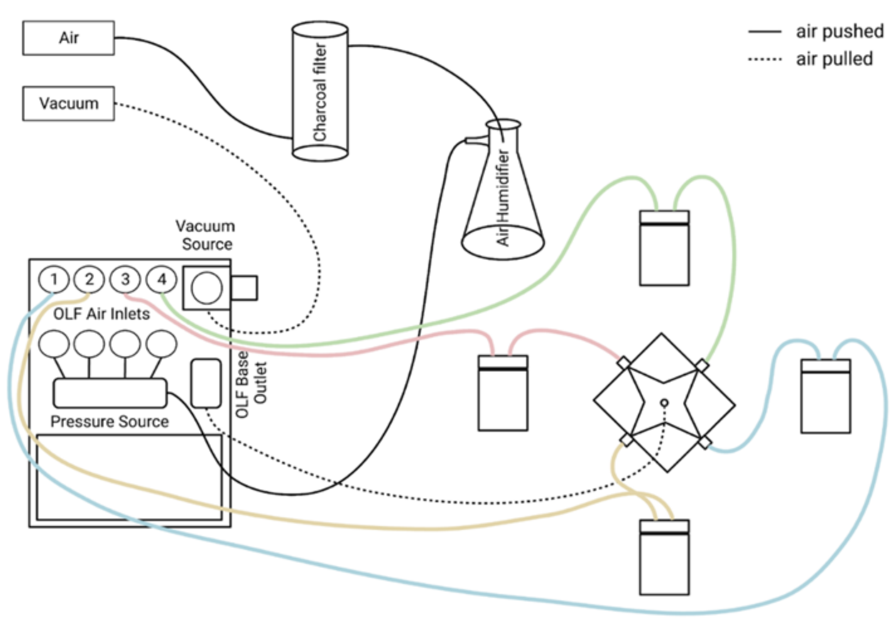
Microbial biological control agents (mBCAs) are emerging as a potentially effective and sustainable way to control disease in many cropping systems. This control can be achieved through diverse modes-of-action, including bioconotrol production of volatile organic compounds (VOCs) and other metabolites that can restrict pathogen establishment and growth. In strawberry crops, mBCAs like the basidiomycete fungus *Aureobasidium pullulans* can be applied during flower bloom to limit post-harvest losses due to infection from *Botrytis cinerea* (grey mold). However, emission of VOCs may have unintended consequences for fruit production, as pollinators like bumblebees are sensitive to scent, including ones released by *A. pullulans* and other flower components. Here, I address if the application of *A. pullulans* as a biocontrol during bloom might affect the scent-mediated interactions between two species of bumblebees (*Bombus impatiens* and *Bombus flavifrons)* and strawberry flowers. To test this question, I performed an olfactometer assay using both bumblebee species and four cultivar/treatment combinations. Those included the two cultivars Royal Royce and Valiant and the treatments of either distilled water or *A. pullulans*. I found that treatment effects on bee preference depended on the bee species, and that cultivar effects on bee preference depended on the treatment. However, bee did not display an innate preference to any one cultivar/treatment combination. Overall, bumblebee responses to *A. pullulans* ranged from neutral to positive. Our data suggest that application during strawberry bloom is likely to have minimal, and perhaps an even positive, effect on bumblebee attraction to flowers.

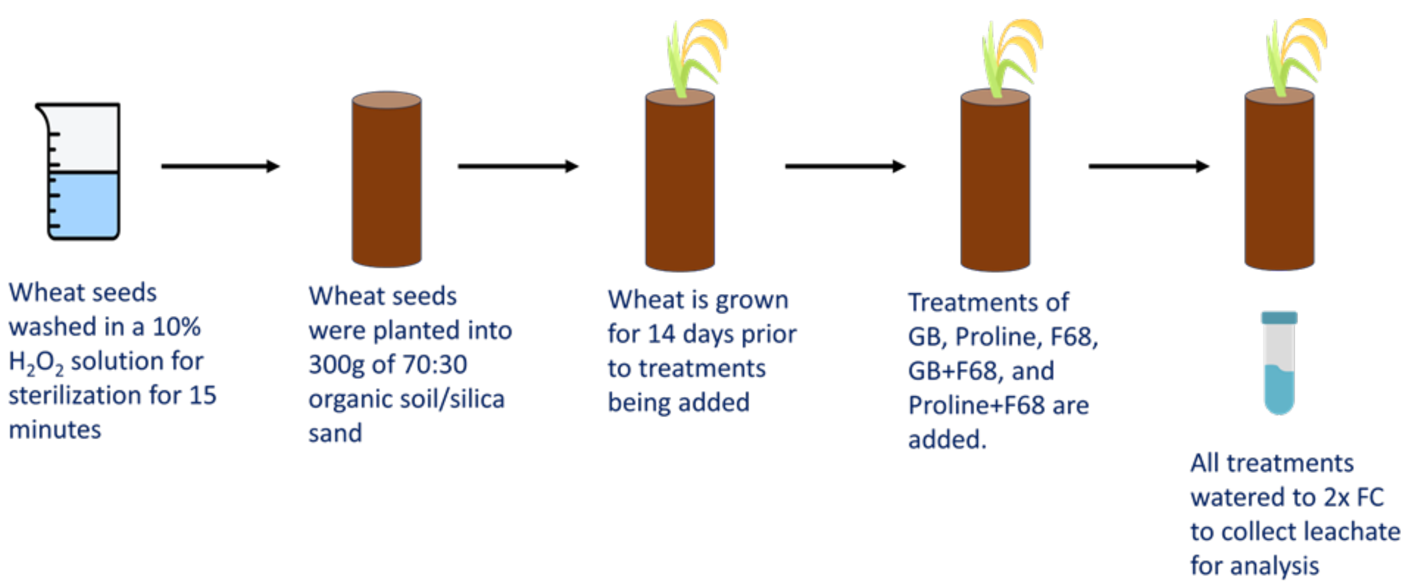


Benefits of Pluronic F68 In Combination of Osmolytes for Water Retention

Adrian Vigil[1], Li-Ting Yen[2,3], Astrid Jacobson[3], David Britt[3]

[1]University of New Mexico, Albuquerque, NM, [2]Utah State University, Logan, UT, [3]National Chung Hsing University, Taichung, Taiwan

The sustainable intensification of agriculture necessitates novel strategies to improve water retention and promote optimal plant growth. In this study, we investigate the combined application of Pluronic F-68, a non-ionic surfactant, with osmolytes proline and glycine betaine as a potential solution to address water scarcity and enhance agricultural productivity. Pluronic F-68, renowned for its unique amphiphilic properties, has been reported to improve water uptake and retention in various systems. This study focuses on characterizing the impact of Pluronic F-68 on water retention in soil. Through soil moisture analysis, we demonstrate that the incorporation of Pluronic F-68 in soil significantly increases water holding capacity, reducing water runoff and evaporation rates. Moreover, the surfactant's ability to improve soil structure enhances root penetration and facilitates nutrient absorption, thereby promoting plant growth.



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