Final Project Report

End-of Project final reports should include final results in layperson's terms (for all audiences) and at least one photo or illustration.

1) Tell us about your project
   - Project Title: Effects of Increased Atmospheric CO_2 and Abiotic Stress on Soybean Performance in the Enviratron

   - Lead PI: Asheesh K. Singh, Steve Whitham, and Lie Tang
     - Co-PIs:

   - Projects year(s): 2021-2024

   - Total amount of funding: $200,000

   - Leveraged/Additional Funding, including federal or private organizations:

2) Project Summary

   Objectives:

   Objective 1: Study of the effects of CO_2 on soybean responses to pathogens in the Enviratron.

   Leads: Whitham, Steve and Tang, Lie.

   - Report of deliverables
     Knowledge of effect of elevated carbon dioxide (CO_2) on bacterial blight (*Pseudomonas syringae* pathovar *glycinea*), soybean mosaic virus, bean pod mottle virus, sudden death syndrome, and seedling root rot (*Pythium sylvaticum*).
     Knowledge of effect of elevated CO_2 on soybean growth and gene expression
     Knowledge of effect of elevated CO_2 on soybean defense responses
   - Non-technical summary (layman's terms)
     The concentration of CO_2 in the atmosphere is steadily rising. When continuous measurements first began in 1958 at the Mauna Loa Observatory in Hawaii, the CO_2 levels were approximately 313 parts per million (ppm). In 2024, the average annual level of CO_2 at this location was 424.6 ppm, a nearly 36% increase over the past 66 years, and it will continue to rise in concert with fossil fuel consumption and land use changes. Increasing CO_2 levels have many effects on plants, such as soybean, some positive and some negative. We were interested in how future elevated CO_2 levels (550 ppm) may be expected to affect the development of soybean diseases. To investigate this, Williams 82 soybean plants were grown in elevated CO_2 and current, ambient CO_2 conditions (419 ppm in May 2022) in

growth chambers, and they were inoculated with bean pod mottle virus, soybean mosaic virus, *Pseudomonas syringae* pathovar *glycinea* (bacterial blight), *Fusarium virguliforme* (sudden death syndrome), and *Pythium sylvaticum* (seedling root and stem rot). Williams 82 plants were less susceptible to bacterial blight, more susceptible to the two viruses and F. virguliforme, and *P. sylvaticum* infectivity was not affected. Our experiments also showed that soybean defenses to bacterial blight were more strongly activated in eCO_2, whereas soybean defenses against the viruses appear to be dampened. We were unable to make any correlations between the status of soybean defenses and the *F. virguliforme* or *P. sylvaticum* infections. These experiments demonstrate that increasing CO_2 can affect the outcomes of soybean disease, causing some to be worse and some better and other unaffected.

1) Conclusions (include observations, solutions, and/or is additional research warranted – limit of 200 words per bullet point)
   - What new knowledge was developed/discovered in the research supported by the ISRC?
     Soybean diseases can be affected by CO_2 levels that are expected in the future - some may be unaffected or decrease in severity, whereas there is potential for others to increase in severity.
     The defense responses of soybeans are affected by CO_2 levels.
   - What are practical applications of the research? How long?
     The results of this research do not have immediate practical applications, but point to new research avenues for the future.
     A lot of attention is focused on temperature and water availability, and rightfully so. However, this research demonstrates that elevated CO_2 levels on their own may have impacts on plant diseases.
     The outcomes warrant additional research 1) to further understand the mechanisms that underlie the altered susceptibility to pathogens in elevated CO_2 conditions and 2) to determine if there are genetic factors that can be used to improve soybean resistance to pathogens in elevated CO_2 environments.
     Additional work is needed to understand how elevated CO_2, temperature, and water availability interact to affect soybean performance.
   - How might this new knowledge/discovery affect the success of positive impact for industry and/or farmers?
     The knowledge provides insight into how soybeans are expected to perform in elevated CO_2 environments.
     The research results suggest that soybean defenses are likely to be altered in the future as CO_2 levels continue to rise, which can affect disease development. This information demonstrates that efforts to genetically adapt soybeans to higher concentrations of CO_2 in the atmosphere should include enhancing disease resistance under those conditions as well.

2) Supporting attachments:

   - Photos/graphs/other graphics

   - Other supporting documents: Publication of findings doi: 10.1111/nph.20364.

3) Other information you want to share (limit of 100 words)
   Leveraged funding from the Plant Sciences Institute to complete the research project.
   Data from this project was used in support of a successful USDA NIFA grant application titled: Machine Learning-Assisted Multimodal Chemical, Biological, Physiological Sensing For Assessing Multiplex Plant Stress States ($591,499)

On a different note, there were some difficulties in utilizing Enviratron rover to automate the image acquisition process, e.g., unreliable communications between the rover and chamber server, and maintenance and manipulation issues. To that end, an upgraded rover design has been developed to integrate an omnidirectional vehicle, a dual-arm configuration, and more capable imaging, ranging, and spectral sensors.

4) List conferences, publications, etc. in which this research was shared
Presented our research as a poster at the American Phytopathological Society meeting in August 2022, Pittsburgh, PA
Presented our research outcomes as a talk and poster at the International Society for Molecular Plant Microbe Interactions meeting in July 2023, Providence, RI
Presented our research at the ISU Research Day hosted by ISRC in 2024
Published the research results in a high-profile plant journal: Bredow, M., Khwanbua, E., Chicowski, A. S., Breitzman, M. W., Qi, Y., Holan, K. L., Liu, P., Graham, M. A., Whitham, S. A. (2025) Elevated CO_2 alters soybean physiology and defense responses, and has disparate effects on susceptibility to diverse microbial pathogens. New Phytologist In press. doi: 10.1111/nph.20364.

Objective 2: Effects of elevated ambient temperatures on soybean gene expression in the Enviratron.

Lead: Singh, A.K.

- Main goals:
    1. Understand the role of native soil microbes in heat tolerance adaptation in soybean
    2. Develop an understanding of key genes, metabolites, and root anatomical traits in soybean for high temperature stress

- Report of deliverables:
  Knowledge of native soil microbes and soybean interactions under prolonged heat stress
  Knowledge of soil microbes behavior under prolonged heat stress

- Non-technical summary (layman's terms)

  Elevated temperatures can disrupt the balance of soil microorganisms and crop growth, leading to altered microbial diversity, community composition, and imbalanced plant development. These changes impact essential soil functions, such as nutrient cycling and organic matter decomposition, ultimately affecting plant health and crop yields. To address these challenges, we studied the effects of high temperatures on soybeans and soil microbiomes using the Enviratron, a climate-controlled facility designed to simulate optimal (28°C/82°F) and elevated (38°C/100°F) temperatures. Our observations revealed that soybeans exposed to higher temperatures progressed through vegetative growth stages more rapidly than those grown at optimal temperatures. However, this accelerated growth did not necessarily translate to improved heat tolerance. We collected tissue samples for gene expression analysis and soil samples to investigate microbial dynamics. The results highlighted that certain heat-sensitive microbes essential for nitrogen fixation were significantly affected by elevated temperatures. Furthermore, below-ground traits, such as root anatomy and nodulation efficiency, were severely impacted by heat stress. Comparisons between plants grown in natural soil and those in sterilized (autoclaved) soil demonstrated that the presence of soil microorganisms influenced gene expression and plant development. This finding underscores the critical role of both plant genetics and soil health in developing strategies to enhance soybean resilience to heat stress. Overall, this research contributes to a deeper understanding of soybean adaptation to high-temperature environments, which is becoming increasingly relevant in the context of current changing climatic patterns.

5) Conclusions (include observations, solutions, and/or is additional research warranted – limit of 200 words per bullet point)

- What new knowledge was developed/discovered in the research supported by the ISRC?
  - This research revealed the effect of heat stress and changes in the soil microbiome to the gene expression of soybean. We found that different genotypes have unique transcriptional responses to heat stress, but there are some conserved genes. We also found that the soil microbiome changes the expression to heat stress, and appears to alleviate some of the symptoms.
  - Soil autoclaving impacts soil chemical properties, increasing phosphorus, sulfur, and manganese levels while decreasing zinc and iron.
  - Autoclaved soil lacks detectable microbial DNA, confirmed through qPCR and soil respiration assays.
  - Significant nodulation was observed at optimal temperatures in non-autoclaved soil treatments.
  - Elevated temperatures significantly influenced microbial activity, root anatomy, and nodulation efficiency.
  - High temperatures and autoclaving did influence the bacterial and fungal diversity and assembly.
  - Experiments revealed the critical role of microbes in soybean growth and stress resilience.

- What are practical applications of the research? How long?
  - The practical applications for this work would take many years still. These applications could be the identification of candidate genes for heat stress tolerance, which could then be bred into commercial cultivars with acceptable yield levels.
  - Informing sustainable soil management practices to optimize microbial diversity to promote soil health in extreme temperatures.
  - Data from this research can guide researchers, with immediate use in experimental designs for follow-up studies.

- How might this new knowledge/discovery affect the success of positive impact for industry and/or farmers?
  - The knowledge gives us greater insight in how soybean adapts to heat stress, and how it can continue to thrive. This opens opportunities for further research into specific molecular pathways and genes to be used to develop heat tolerant soybean. This is further coupled with the evidence that the soil microbiome contributes to the heat stress response, and can aid in alleviating the stress.
  - Promotes better soil management practices, leveraging microbes for improved crop health and productivity.
  - Provides a framework for integrating microbial health into agricultural practices, benefiting farmers facing heat stress challenges.
  - Fostering collaboration with industry partners to understand microbial-soil-plant interactions, for driving innovations in crop production and sustainability.

6) Supporting attachments:

- Photos/graphs/other graphics

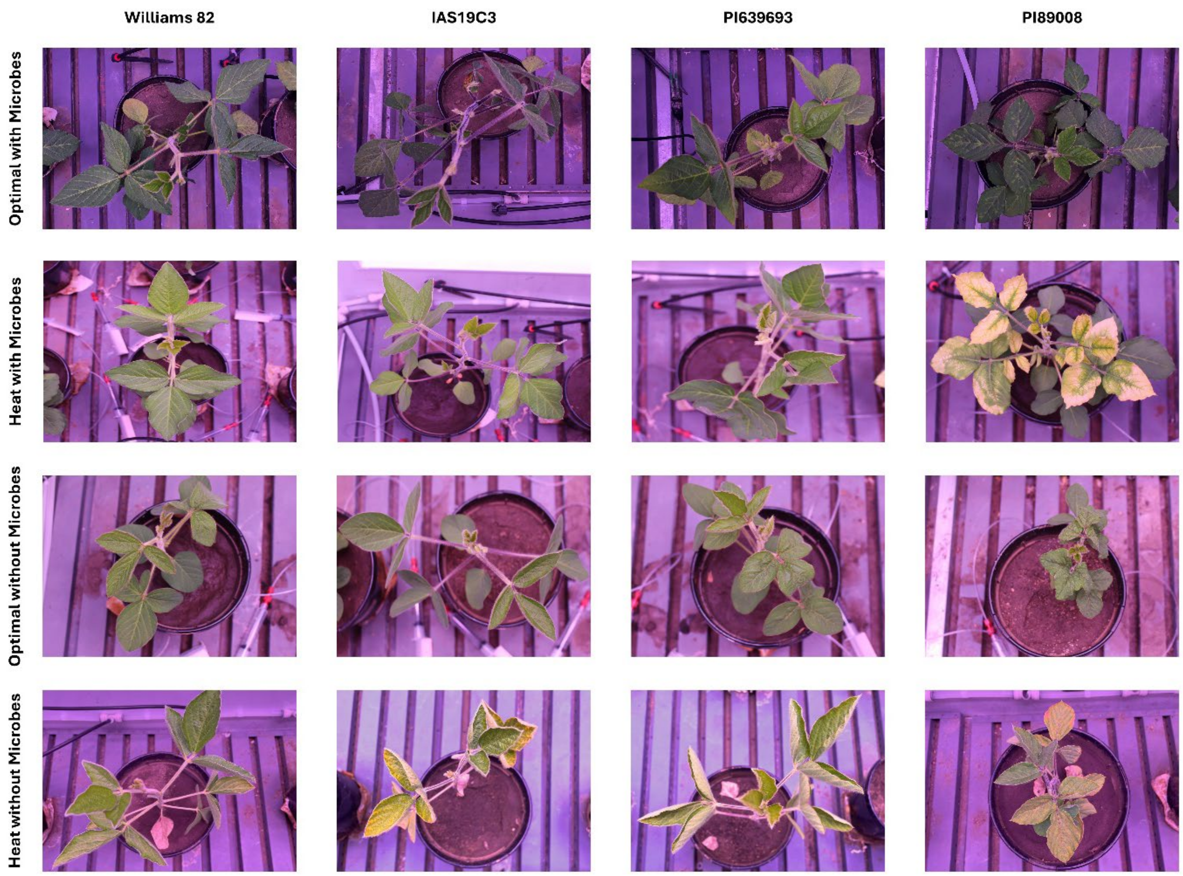

Figure 1. Images of the four soybean genotypes in the four different treatments (factorial combination of heat stress and soil microbiome). Williams 82 was the control genotype, IAS19C3 is the elite genotype, PI639693 is the heat tolerant genotype, and PI89008 is the heat susceptible genotype.

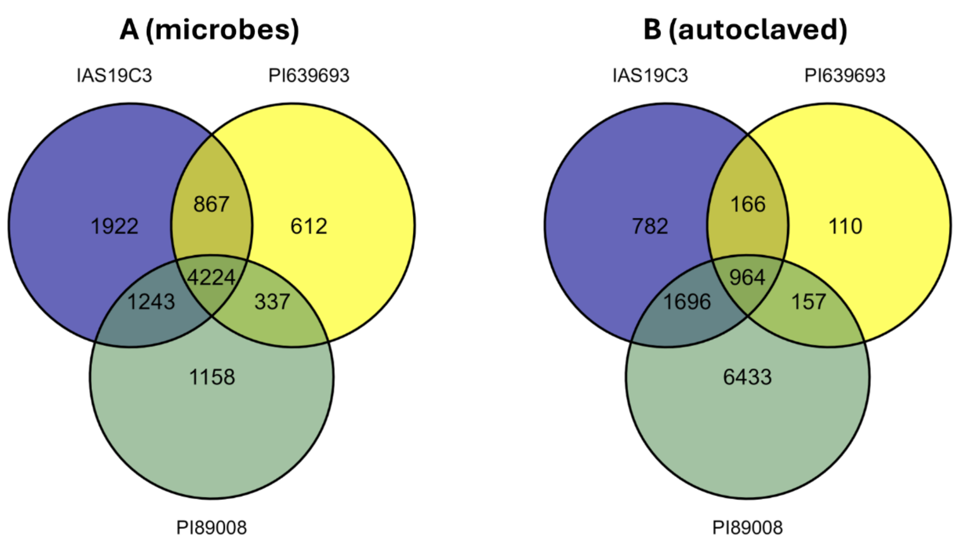

Figure 1. Venn diagrams of differentially expressed genes (DEGs) due to the effect of heat stress and the genotype, when grown in soil with a native soil microbiome (A) and when grown in autoclaved soil with no soil

microbiome (B). This shows the unique responses each genotype had in response to heat, as well as how this changed based on soil microbiome conditions. From the 4224 and 964 genes that overlapped for all three genotypes in their respective soils, only 83 were shared across the soil microbiome conditions.

Figure 3. Rate of nodulation is increased with microbes under optimal growing conditions

7) List conferences, publications, etc. in which this research was shared

   a. ASA-CSSA-SSSA Annual Meeting (October 30, 2023)
   b. R.F. Baker Plant Breeding Symposium (March 22, 2024)
   c. Presented our research outcomes in ASA-CSSA-SSSA meeting in 2023
   d. Showcased our research in the ISU Research Day hosted by ISRC 2024
   e. Presented our research at the International Phytobiomes Conference 2024
   f. Published a bioRxiv pre-print (https://doi.org/10.1101/2024.11.04.620947)
   g. Peer-reviewed papers (2) are under development