ISRC Project Update

Project updates should include what steps have been/will be taken and where the researcher is at in the process.

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: Dr. Asheesh K. Singh
        - Co-PIs: Dr. Steven A. Whitham, Dr. Lie Tang
    - Projects year(s): 2 years (10/1/2021 – 9/30/2023)
    - Total amount of funding: $200,000
    - Leveraged/Additional Funding, including federal or private organizations: n/a

2) Project Summary

    The goals of this project are to investigate the performance of soybean under future climate scenarios with respect to disease development and abiotic stress tolerance. The long-term goal of this research is to inform forward-looking breeding approaches to develop soybean germplasm well-suited for future production environments.

    - Objectives:
        1. Study of the effects of CO2 on soybean responses to pathogens in the Enviratron. (Leads: Whitham, S.A. and Tang, L.)
        2. Effects of elevated ambient temperatures on soybean gene expression in the Enviratron. (Lead: Singh, A.K.)
            a. Understand the role of native soil microbes in heat tolerance adaptation in soybean
            b. Develop an understanding of key genes, metabolites, and root anatomical traits in soybean for high temperature stress
    - Deliverables:
        1. Understanding of how increased atmospheric CO2 affects development of diseases caused by viruses, bacteria, and fungi in soybean.
        2. Image analysis approaches for automated detection of diseased and heat stressed soybean plants.
        3. Heat tolerance screening protocols will be streamlined

4. 2-3 open access journal publications will be developed by the end of second year of this project that will report on the key genes, metabolites, and root anatomical traits in heat stress.

- Benefit to Soybean Farmers: A greater understanding of how increased atmospheric CO_2 affects development of diseases caused by viruses, bacteria, and fungi in soybean will provide insight into future disease management scenarios. A greater understanding of the genetics for heat stress tolerance in soybeans will allow breeders to develop soybean varieties that can withstand these temperatures. The work in these two objectives will benefit future farmers as it ensures that they can maintain or increase their yields even in more adverse and different conditions expected in the future.

3) Progress Update (what steps have been taken/initial set up or early findings)

Objective 1: Study of the effects of CO_2 on soybean responses to pathogens in the Enviratron.
For all experiments, conditions were used that simulate an average mid-June day in Iowa with either 419 ppm or 550 ppm CO_2, and soybean cultivar Williams 82 was used for all experiments.

a. Establish baseline physiology of soybean plants growing at 419 ppm versus 550 ppm CO_2 in Enviratron growth chambers.

i. shoot biomass (wet and dry)
ii. plant height
iii. stomatal conductance (gas exchange occurring through leaf stomata)
iv. stomatal density (number of stomata per unit leaf area)
v. stomatal aperture (width of stomatal opening)
vi. photosynthetic activity
vii. RNA sequencing to determine effects of elevated CO_2 on soybean gene expression. We are particularly interested in the effects of elevated CO2 on the expression of defense-related genes. RNA was extracted from plants and submitted to the ISU DNA Facility for sequencing. Data analysis is in progress.

For this objective, most data have been collected and analyses are in progress. In general terms, we find that plants grown under 419 ppm and 550 ppm CO_2 are responding as we expect based on the literature. Plants grown at the higher CO_2 level develop more biomass, are taller, and have greater photosynthetic activity at 35 days after sowing. In contrast, stomatal conductance and stomatal density is reduced. Stomatal aperture measurements are in progress.

b. Determine effects of elevated CO2 on five different pathogens interacting with soybean

i. Bean pod mottle virus (BPMV, bean pod mottle)
ii. Soybean mosaic virus (SMV, soybean mosaic disease)
iii. *Pseudomonas syringae* pathovar glycinea (Psg, bacterial blight)
iv. *Fusarium virguliforme* (sudden death syndrome)
v. *Pythium sylvaticum* (damping off)

vi. response to flg22 elicitor. Flg22 is a small peptide that is used to trigger basic soybean immune responses.

For this objective, we spent significant time in the first year to establish inoculation conditions for Psg, *F. virguliforme*, *P. sylvaticum*, and the flg22 treatment under the required growth conditions. We also developed required inoculum for BPMV and SMV experiments, and a standard rub-inoculation procedure was used for these viruses. All inoculations have been performed and samples have been collected for all the treatments or is in the process of being collected in repeat experiments. We are in the middle of data analyses, so no final conclusions are presented here. Collected data include biomass, disease ratings over time and at experiment end points, and when possible quantification of the pathogen. In some experiments, we are also collecting RNA samples for virus quantification or analysis of soybean gene expression.

In general terms, young soybean plants appear to be more resistant to the bacterial pathogen, and we are trying to understand why this is the case, because there are at least two possibilities. The first is physical: there are fewer stomata and less stomatal conductance, which suggests that stomata are not open as wide at 550 ppm as at 419 ppm. Therefore, there are reduced sites for Psg to enter the plant. The second is that we also observed that flg22 and bacteria inoculation trigger a much stronger defense response in plants grown at 550 ppm CO_2, which suggests enhanced defense to bacteria at the higher CO_2 level. In contrast to bacteria, the plants grown at 550 ppm appear to develop disease faster when infected by SMV or BPMV. We are in the process of quantifying virus levels to determine if the viruses accumulate to higher levels in plants grown at 550 ppm. We are still analyzing data from the *F. virguliforme* and *P. sylvaticum* experiments.

Objective 2: Effects of elevated ambient temperatures on soybean gene expression in the Enviratron. After thorough project planning, soil collection and preparation were started in the winter of 2021-2022. The initial experiment was planted in the Enviratron in April 2022, however due to soil compaction and drainage problems, this experiment had to be terminated within two of the planned five weeks. This resulted in another round of soil collection and preparation. A new experiment was planted in June 2022, and two of the four treatments were successfully sampled. During this time, visible symptoms of stress were noted in certain genotypes in the elevated temperature chambers (Fig. 1). The remaining two treatments could not be sampled due to sudden chamber malfunctions within the last days of the experiment. Because of this, the two treatments were planted again in July, and were successfully sampled five weeks later. Please see pictures below of sampling day (Fig. 2).

For our experiment, we have used four soybean genotypes (Williams 82, IAS 19C3, PI 639693, and PI 89008) with (non-autoclaved) and without (autoclaved) microbes under high (day: 38^{o} C for 16 hrs and dark: 28^{o} C for 8 hrs) and optimum (day: 28^{o} C for 16 hrs and dark: 21^{o} C for 8 hrs) temperatures with 60% relative humidity and current ambient CO_2 levels (400 ppm). We collected tissues for DNA, RNA, root exudate, and anatomical studies. Figure 3 shows the autoclaved soil does not have any DNA in it, meaning a load of available microbes was negligible or not detected; we have confirmed the same with qPCR and soil respiration assays (Fig. 4). We have observed significant respiration differences between autoclaved and non-autoclaved soils; non-autoclaved soils showed a fourfold cumulative CO_2 flux difference compared to autoclaved soils (Fig. 4). Autoclaving did not change the physical property of the soil (Table 1 and Fig. 5); however, it changed the chemical properties of the soil, the level of phosphorous, sulfur, and manganese were increased significantly, and zinc and iron levels were going down, and other factors were unaffected. We have observed significant nodulation in the optimum temperatures' treatment with

microbes (non-autoclaved soil) (Fig. 6). We are planning on performing the follow-up experiment for the same.

In August and September, DNA and RNA extractions were successfully completed for all treatments. Rhizosphere soil DNA was extracted using Qiagen's DNeasy power soil kit. Following these extractions, much of the fall of 2022 was taken up with necessary quality control and standardization of the extracted DNA and RNA before submission for sequencing (Fig. 7). The high-quality DNA was used for 16S and ITS library preparation and MiSeq sequencing. For the bacterial 16S hypervariable region V_4 and for the fungal ITS1 region, the primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) and ITS1f (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) were used, respectively. Libraries were sequenced using paired end 250 cycle MiSeq (500 cycles total). DNA and RNA samples were submitted for sequencing in November, and sequencing was completed by the ISU DNA Facility in late December. Data is now in hand and preliminary data exploration has been started. After this, full data analysis will be completed, and preparation of a journal manuscript will begin.

Additionally, due to observations that were noted during the experiment, a small project was planned and completed as an undergraduate research project. This has allowed a student interested in pursuing plant breeding related research to gain some practical experience in research and start developing her own research skills. While this is not a specific goal of this project, being able to develop the next generation of scientists is always a benefit.

4) Supporting attachments:

- Photos/graphs/other graphics

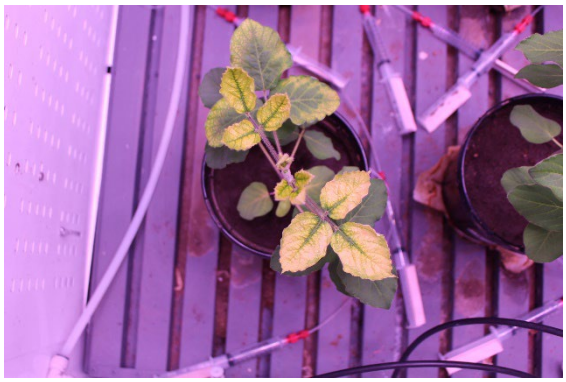

Figure 1. Specific genotype displaying chlorosis in heat chambers.

Figure 2. Dinakaran Elango and Liza Van der Laan collecting tissue samples in the Enviratron.

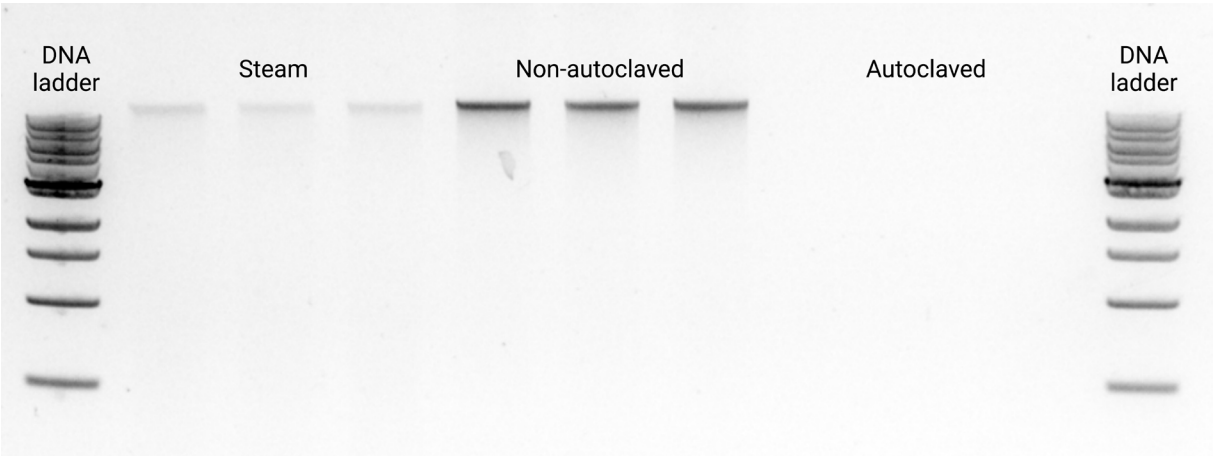

Figure 3. Presence of DNA in lightly autoclaved (steam), non-autoclaved, and autoclaved soils.

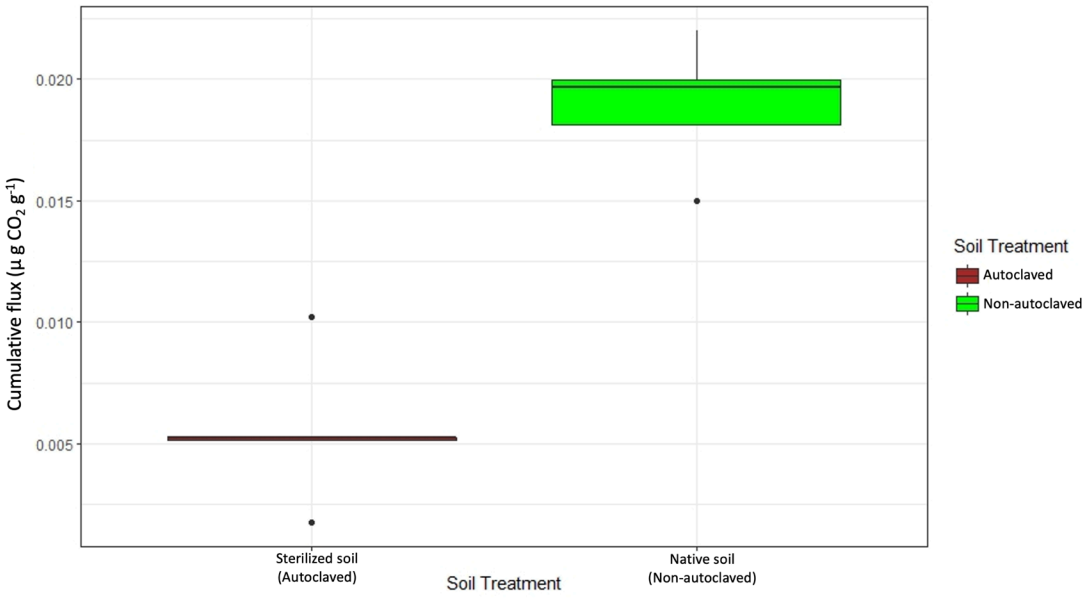

Figure 4. Level of soil respiration activities in autoclaved and non-autoclaved soils

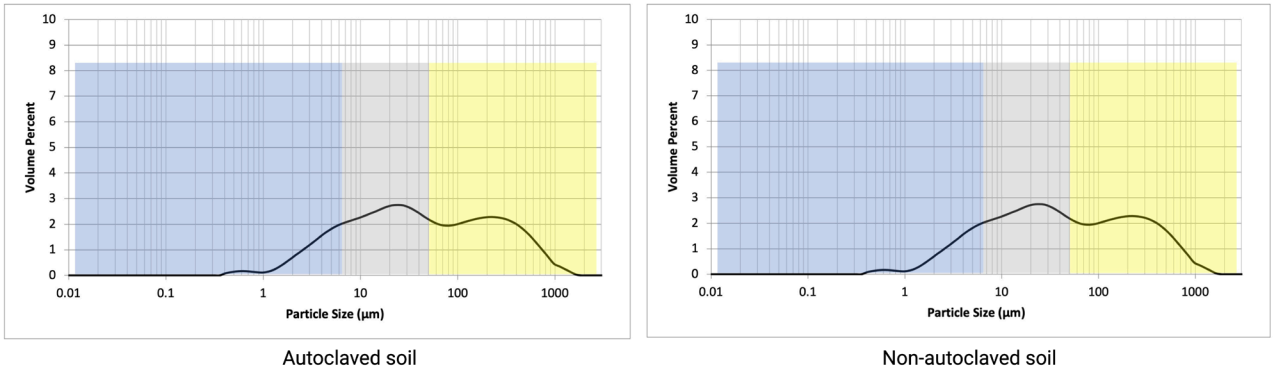

Figure 5. Physical properties of the autoclaved and non-autoclaved soil types

Figure 6. Rate of nodulation is increased with microbes under optimal growing conditions

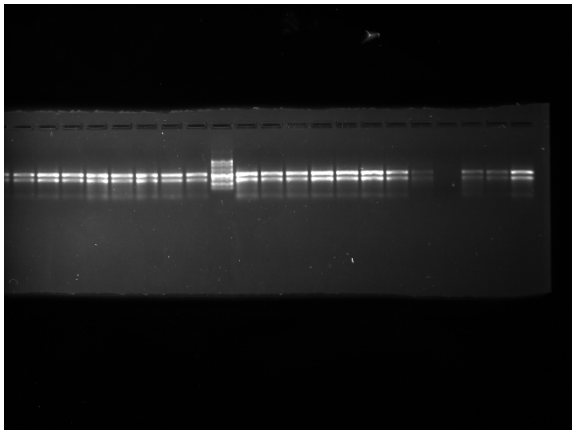

Figure 7. Gel of extracted RNA for quality check.

Table 1. Physical properties of the autoclaved and non-autoclaved soils (Soil texture class: Loamy)

Soil treatment	Clay	Silt	Sand	Very fine sand	Fine sand	Medium sand	Coarse sand	Very coarse sand
					µm			
Autoclaved	22.05	31.44	46.35	14.25	12.05	12.20	6.81	1.05
Non-autoclaved	22.11	32.46	45.31	14.66	11.81	11.64	6.31	0.89