Fall 2024
Honors Research Fellowship Reports

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Major: Biomedical Engineering

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Role of the Pregnane X Receptor in Wound Healing and Macrophage Metabolism

Introduction:

Diabetic foot ulcers are a common complication experienced by diabetics, affecting approximately 25% of patients. *Diabetes mellitus* patients have been shown to have elevated levels of prevalent organochlorine pesticide metabolites. These metabolites can bind to a variety of receptors in the body to have unforeseen consequences. The pregnane x receptor (PXR) is a xenobiotic sensing receptor that regulates the expression of genes encoding drug-metabolizing enzymes, drug transporters, and cellular metabolism. Study of the PXR is useful for defining the effect of the receptor on pharmacokinetics, drug toxicity, and efficacy. However, the role of the PXR in wound healing remains largely unstudied. The present study was designed to define the role of the PXR in wound healing and to determine if sex differences in wound healing exist. Additionally, the PXR was investigated in macrophage function and metabolism of bone marrow-derived macrophages (BMDM) given the critical role of macrophages in wound healing.

Methods:

To define the role of the PXR in wound healing, *in vitro* studies were conducted in four groups of mice: male pregnane X receptor knockout (PXR KO) mice, female PXR KO mice, male C57BL/6J mice, and female C57BL/6J mice. Animals were subjected to two 12-hour

ischemia-reperfusion cycles to simulate the development of foot ulcers. Once wounding was complete, digital images were captured on days 1, 4, 8, and 12 post-wounding. Wound area was quantified at each time point and plotted as a function of time to track the rate of wound resolution in each study group. Data was analyzed using a repeated measures two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test to determine the total effect of PXR on wound healing as well as gender-specific effects.

To investigate the role of PXR in macrophage function and BMDM metabolism, femoral and tibial bone marrow were collected from male and female C57BL/6J mice 16-20 weeks of age. Cells were isolated from bone marrow and differentiated into macrophages. Following differentiation, BMDM were treated with DMSO (vehicle), pregnenolone 16α-carbonitrile (PCN; PXR agonist), vehicle + lipopolysaccharide (LPS) + interferon-gamma (IFN-γ), or PCN + LPS + IFN- γ in serum-free DMEM for 24 hours. Following the treatment period, cell media was collected and subjected to metabolomic analysis. Additionally, cell extracts were prepared for metabolomic analysis. Relative levels of itaconate, citrate, succinic acid, and pyruvate were quantified and compared between treatment groups within sexes as well as between sexes using one-way ANOVA with Tukey's multiple comparisons test and two-way ANOVA with Tukey's post hoc for pairwise comparisons, respectively.

Results:

Wound healing in female mice was found to be PXR-independent, while healing in male mice was found to be PXR-dependent. In control mice, females had a significantly slower rate of wound resolution at intermediate time points but ultimately achieved the same level of wound resolution as male mice. In PXR KO mice, total wound resolution was significantly less in male mice, indicating the important role of the receptor in both overall wound resolution and healing

rate in males. Thus, enhanced PXR activation could offer a promising therapeutic strategy for improving wound resolution outcomes in male patients.

In select BMDM treatment groups, citrate and itaconate levels were significantly higher in female BMDM media than male media, suggesting a pro-inflammatory, pro-healing phenotype in female mice. Itaconate levels were found to be significantly higher in BMDM male and female cell extracts treated with vehicle + LPS and PCN + LPS, suggesting that itaconate production increases in response to pro-inflammatory signaling from LPS regardless of PXR activation status. Stimulation with LPS significantly increased succinic acid levels in vehicle male cell extracts, while this finding was absent from female cell extracts. Sex differences were particularly notable in cell extract pyruvate levels with significantly higher pyruvate levels observed in female cell extracts subjected to vehicle, PCN, and vehicle + LPS treatment. Differences in immunometabolism between male and female mice may offer insights into sex-dependent inflammatory profiles and wound resolution rates. However, findings indicate limited effects of PXR activation regardless of LPS stimulation, suggesting the PXR may play a limited role in augmenting BMDM cellular metabolism, particularly the TCA cycle.

Significance/Future Directions:

In the future, activation/inactivation of the PXR may serve as a valuable therapeutic target clinically. Given the sex-dependent roles of the receptor in wound resolution along with the differential effects of the receptor observed in select stages of wound formation, targeting of the PXR should be considered on a case-by-case basis. For those patients receiving treatment early in the wound development process, administration of a PXR antagonist may be beneficial to reduce initial wound size. However, for patients receiving treatment after a wound has fully developed, administration of a PXR antagonist may be detrimental, resulting in a reduced rate of

wound healing. In male patients, PXR agonists may enhance the rate of wound resolution, thereby ameliorating worse healing outcomes seen in males. While further study is required to understand the stage-specific and sex-specific effects of the PXR, preliminary findings indicate that the receptor may be a valuable target clinically to reduce the risk of chronic wound development, lower wound-associated mortality, and improve overall patient outcomes.

Note:

Complete details and results of this project may be found in the following manuscript, available for download through Mississippi State University's Institutional Repository:

> Thompson, Annamarie L., "Role of the Pregnane X Receptor in Wound Healing and Macrophage Metabolism" (2025). *Honors Theses*. 142. https://scholarsjunction.msstate.edu/honorstheses/142

The author expresses gratitude to the Judy and Bobby Shackouls Honors College for their financial support of this work through a Shackouls Honors College Undergraduate Research Fellowship. Funds from the fellowship were used to purchase laboratory supplies for cell culture and metabolomic analysis as well as a GraphPad Prism student subscription.

Honors Undergraduate Research Fellowship Report

Name: Kayla Young

Faculty Advisor: Dr. Richard Baird

Project Title: Assessing the Impact of Drought and *Macrophomina phaseolina* Infection on the Soybean Microbiome

During the 2024-2025 academic year, I had the privilege to gain undergraduate research experience in Dr. Richard Baird and Hannah Purcha's lab.

Introduction

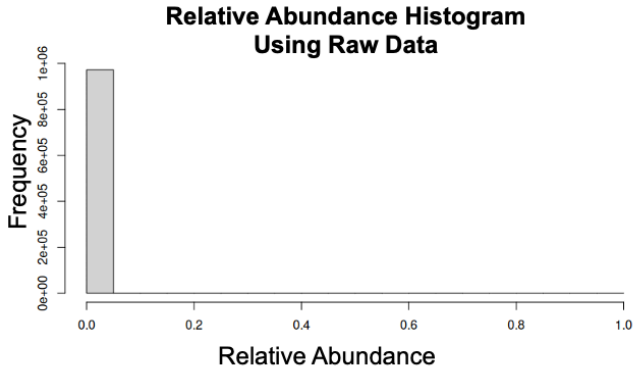
Charcoal rot, caused by *Macrophomina phaseolina* (MP), significantly impacts soybean yields in the US, particularly under stress like drought. Current control methods offer limited success, and no resistant soybean varieties exist. However, asymptomatic plants often grow alongside infected ones, suggesting an epigenetic factor to susceptibility. Research indicates that endophytic microbial communities differ between healthy and infected plants. Therefore, we hypothesize that the endophytic community of a plant impacts its ability to survive MP and/or drought stress. This research project investigates this hypothesis by comparing the metagenomic DNA extracted from soybean plants subjected to four treatments (MP, drought, MP and drought, and a control) to evaluate how these stressors alter the microbial community.

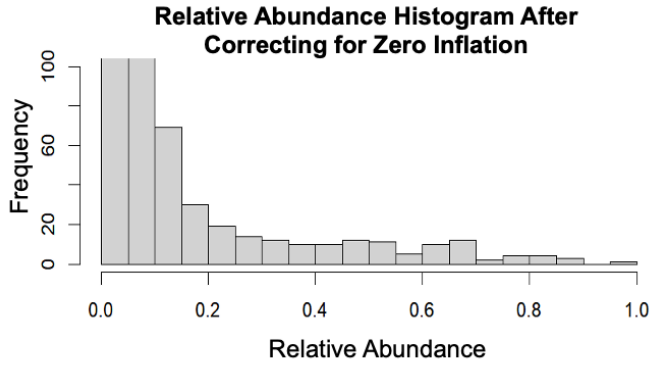
Materials and Methods

The plants were planted on 6/6/2023 using the seed: Progeny P4604 XFS. The harvest dates were 7/11, 8/4, and 8/24. There were 30 pots per treatment for a total of 120 samples. The table below shows the treatment variations for the plants.

Treatment	MP	Drought
1	-	-
2	+	-
3	-	+
4	+	+

Results





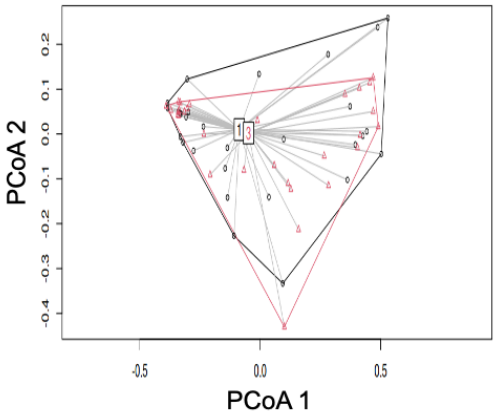
Kruskal-Wallis Rank Sum Comparing Relative Abundance by Treatment

χ^2	df	*p*-value
328.09	3	< .001

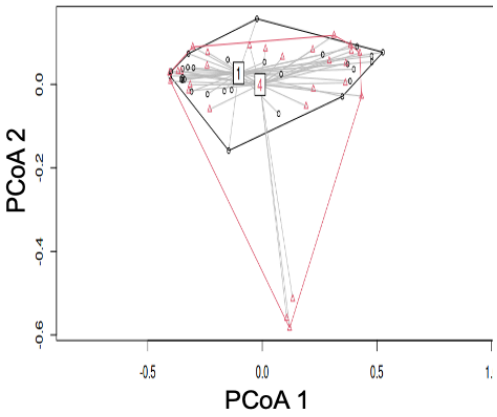
Pairwise Comparisons Using Wilcoxon Rank Sum Test with Continuity Correction

	1	2	3
2	<.001	-	-
3	0.04	<.001	-
4	<.001	<.001	<.001

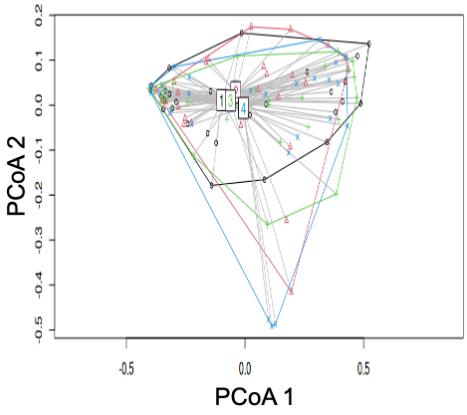
Beta Diversity Distance Matrices Comparing Treatments 1 and 3



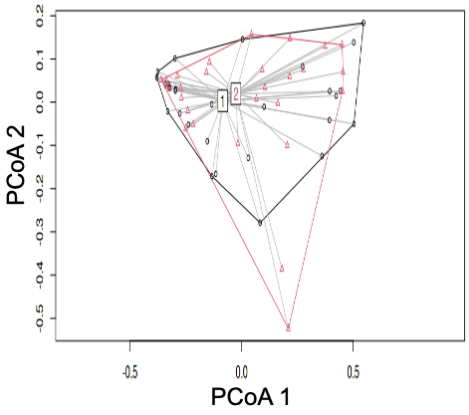
Beta Diversity Distance Matrices Comparing Treatments 1 and 4



Beta Diversity Distance Matrices Comparing All Treatments



Beta Diversity Distance Matrices Comparing Treatments 1 and 2



Plant Pathogenic Fungal Species ANOVA

	df	Sum Sq	Mean Sq	F	*p*-value
Treatment	1.00	0.00	1.5E-05	1.45	0.23
Residuals	39328.00	0.40	1.0E-05	-	-

Pairwise Comparisons of Plant Pathogenic Fungi Abundance

	1	2	3
2	0.22	-	-
3	< .001	0.27	-
4	< .001	0.27	0.89

Endophytic Fungal Species ANOVA

	df	Sum Sq	Mean Sq	F	*p*-value
Treatment		1.0E-08	9.9E-09	0.06	0.81
Residuals	1822	3.1E-04	1.7E-07	-	-

Pairwise Comparisons of Endophytic Fungi Abundance

	1	2	3
2	1.00	-	-
3	1.00	1.00	-
4	0.84	0.56	0.93

Epiphytic Fungal Species ANOVA

	df	Sum Sq	Mean Sq	F	*p*-value
Treatment	1	9.0E-09	9.4E-09	20.18	< .001
Residuals	10144	4.7E-06	4.7E-10	-	-

Pairwise Comparisons of Endophytic Fungi Abundance

	1	2	3
2	1.00	-	-
3	0.07	0.07	-
4	0.07	0.07	1.00

Discussion/Conclusion

The data is significantly zero inflated, which poses difficulties when attempting to evaluate the differences in microbiome composition between the treatments. Using a zero-inflated negative binomial model along with the non-parametric Kruskal-Wallis tests, we found statistically significant differences in the microbiome composition between all treatments. Comparing the beta diversity distance matrices, the treatments had similar beta diversity overall, but the MP-positive treatments were more dissimilar to the control group. When evaluating the categories of fungi present, pairwise comparisons did not fully elucidate the differences between the treatments, and further investigation is warranted. Conducting these analyses on samples from a field trial. Correlating the microbial and metabolic shifts observed in the samples in the study. Investigating additional statistical models that are better suited to zero-inflated data.

Additional Research

In addition to working with Dr. Baird, I also was able to do some work with Dr. Galen Collins. In his experiment we were studying a mutation of the proteosome that is found in several kids with neurodevelopmental disorders. These patients are heterozygous for a P320R mutation in the gene PSMC5. Dr. Collins has cell lines that are PSMC5 P320R/WT and PSMC5 P320R/P320R (Het and Homo, respectively). Wild-type PSMC5 has been expressed in these to rescue the genes. The objective is to analyze whether these mutations affect the degradation rate of two model proteins (GFP-CL1 and INSIG1-GFP).

The work I did with Dr. Collins was only the first step of the extensive experiment: determine what DNA and transfection reagent concentration is needed to detect these proteins. Our goal

was to experimentally transfect cells with plasmids with these GFP-fusion proteins to figure out what concentrations to use in the BE(2)M17 cells.

Over several weeks we conducted the following procedures: detached and counted the cells, seeded for the experiment, transfected the cells and collected, lyse cells to extract proteins, run Bradford assay to determine protein concentration, make gel and run the gel electrophoresis for protein separation, transfer protein onto membrane, block the membrane with 5% milk, incubate with antibodies, then detect and analyze.

Fellowship Takeaways

The Honors Fellowship was highly beneficial to my research experience. The fellowship funded my hourly salary while working in the lab to allow me to give my full focus to building my research skills. The skills I have gained will ensure my readiness for graduate school that I will begin this fall.

Fall 2024 Honors Undergraduate Research Fellowship Report

Name: Tanner Riley Marlow

Faculty Advisor: Dr. Richard Baird

Project Title: Evaluating the Impact of *Macrophomina phaseolina* on the Metabolome of soybeans Grown in Field Conditions.

I had the privilege to work as an undergraduate research assistant under Dr. Richard Baird and PhD graduate student Hannah Purcha for the 2024 fall semester evaluating the impact of *Macrophomina phaseolina* (Mp) on the metabolome of soybeans grown in field conditions.

Project Introduction:

Soybeans (*Glycine max*) have numerous uses including food, feed, oils, and biofuels. Total value of soybean production in the United States was nearly $39 billion. Consisting of 4.54 billion bushels in 2019 (soystats.com/2019-soystats/) this makes the United States one of the three world leaders in soybean production. However, charcoal rot disease of soybean, caused by the soilborne fungus Mp, is a major disease that resulted in the loss of more than 200 million bushels across 28 US states and Ontario, Canada from 2010-2014. The fungus is known to cause root/stalk rot or charcoal rot disease in more than 500 plant species, including many agricultural and horticultural species. Charcoal rot disease is endemic in many southern soybean growing areas where summers are relatively dry, or irrigation is needed. In soybean, the disease causes a general root rot, premature yellowing and defoliation of leaves through attacking lower parts of

the plant such as the lower stem and roots. There are no fungicides available for effective disease control, and excellent genetic resistance is not available in cultivars. Research on biologically based management of plant diseases is in demand due to the lack of effective control methods.

Purpose and Hypothesis:

The goals of this research program are to understand the fundamental basis of plant-microbe interactions in the presence of plant pathogens and to develop biologically based approaches to plant disease management. This disease spreads very quickly and effectively in a field; however, in an affected area, there are some soybean plants that remain healthy. The surviving plants are identical genetically and grow under the same soil and weather conditions and have the same pathogen pressure as the infected plants. We hypothesize this plant protection is due to endophytic organisms that play critical roles in plant resistance to the disease, through either biological priming or direct antagonism against the pathogen.

Objective and Significance:

To test this hypothesis, specific objectives of this project are **(1)** Characterization of endophytic bacterial and fungal communities using culture-dependent methods; **(2)** Community analyses of fungal, bacterial endophytes, and metabolomic changes associated with plant disease development; **(3)** Evaluation of pathogen-endophyte-plant interactions; and **(4)** Elucidation of the mechanisms of interactions between endophytic microbes and plant growth and disease development. Community analyses will be used to identify and select cultures for biocontrol studies. This research will improve our current understanding of the role of endophytic functionality in plant disease resistance in an agriculturally relevant soybean production system.

The findings of this research will elucidate plant-pathogen-microbe interaction mechanisms and help to develop feasible biologically based approaches for improved plant production and IPM.

Materials and Methods:

For materials and methods, the first step was collecting samples. The soybeans were collected from the Mississippi State University Delta Extension Center in Stoneville, MS. Plants from charcoal rot infested fields will be collected from September – October. Rows of two standard soybean cultivars, Delta Grow 47XF90STS and Dyna-Gro S48XF35, were evaluated. Within each row, relatively healthy and severely diseased soybeans that were growing beside each other were harvested; a total of 10 healthy and 10 dying plants per cultivar per row were collected for a total of 40 samples. After collection, the foliar tissue was stored at -80 ºC before their metabolites were extracted using the protocol developed by Kim et al., 2010. The plants will be processed in the fall for nuclear magnetic resonance (NMR) preparation and data collection through December 2024. The metabolite extracts were evaluated on the 500 MHz Bruker NMR, and the spectra were analyzed using the Chenomx NMR Suite (v9). The metabolic data was processed using the MetaboAnalyst (v6.0) platform. In late fall 2024 and early spring 2025, the endophytic data through whole-community analyses (MiSeq Sequencing) will be collected from the same plant tissues used for NMR analysis. Multivariate analyses will be conducted during late summer-early spring, 2025.

During the 2024 fall semester, I engaged in the initial portion of the research process. After proper lab training and preparation, this process included three main takeaways. The first was conducting soybean disease ratings based on potential presence of Mp and the length of the soybean specimen from the first node to the top of the plant. Secondly, I started molecular

microbial community identifications with associated Mp disease infections from field variety sampling. Thirdly, final training for metabolomic analyses was by preparing samples for nuclear magnetic resonance (NMR), learning to extract metabolites from tissues and began running tissue using the NMR facility in the Hand Chemistry building to obtain secondary metabolite data associated with the plant disease process.

Discussion/Conclusion:

This research process took the 2024 fall semester and the 2025 spring semester to obtain data. Therefore, data was not collected for the fall of 2024 semester. The data and graphs will be crafted and explained in the 2025 spring semester report.

Fellowship Takeaways and Acknowledgements:

During my time in Dr. Baird's lab for the fall 2024 semester, I learned an extreme amount regarding lab procedures, how to conduct myself in a professional lab setting, how to communicate with superiors about what I was doing in the lab, and learning procedures such as NMR and DNA extraction. My time in the lab for the fall of 2024 helped me to understand to a greater extent the responsibility of lab workers and the importance of transparent communication to those coming into working the lab so that progress on the projects can be made. Overall, the main takeaway I got during the fall of 2024 semester was the abilities obtained in pursuit of the results of the study such as DNA extraction, samples analysis, and NMR preparation.

Regarding funding, this research experience would not have been possible if it were not for the funding of the honors college. NMR tubes are extremely expensive, and the $2,000 obtained through this fellowship allowed Dr. Baird's lab to buy this material and use the NMR lab in the Hand Building. Without the support of the Shackouls honors college, I would not have

been able to be a part of this research experience, and for that I am extremely grateful for the generosity of the college to help fund this initiative.