Spring 2025
Honors Research Fellowship Reports

Director:

Dr. Anastasia Elder

Edited by:

Morgan Elmore

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Spring 2025 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's for the 2025 spring semester evaluating the impact of *Macrophomina phaseolina* (Mp) on the metabolome of soybeans grown in field conditions.

Project Introduction:

Macrophomina phaseolina (MP) is a generalist, soil-borne fungus that causes a disease known as charcoal rot in many types of crops, including but not limited to pulses, cotton, corn, and melons. One crop that is incurring significant losses due to this pathogen are soybeans, a major crop in the southeastern United States. In 2023 alone, charcoal rot resulted in an estimated loss of 9,485,000 bushels of soybean in just the United States. MP's destructive capacity is exacerbated by hot and dry conditions, which are becoming more common across many centers of soybean production. Previous research in the Baird Lab has shown that MP and drought conditions significantly alter the metabolome of soybeans, but these findings utilized highly controlled greenhouse conditions. However, for this study, samples of two different varieties (Delta Grow 47XF90STS and Dyna-Gro S48XF35) were collected from the Mississippi State

Delta Research Extension Center in Stoneville. The metabolites were extracted from the foliar tissue of these plants and were analyzed using the 500 MHz NMR platform. Evaluations of the metabolic profiles, root disease severity, and other growth parameters were conducted, and the results are presented in the poster.

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 2025 spring semester, I pursued community analyses of fungal/bacterial endophytes and metabolomic changes associated with plant disease development and evaluated pathogen-endophyte-plant interactions. These samples are completed for the NMR and DNA analyses; however, the results for the DNA will come in later this year. The NMR data was used

to craft the figures listed below. In the spring semester, the preparation for bioinformatics analysis of microbial presence and metabolites, supporting the training of new students in the lab, and presenting at the undergraduate spring research symposium was all accomplished during this semester.

Results/Discussion:

The results were acquired in the spring 2025 semester prior to the spring symposium. To predict a set of dependent variables from the large set of independent variables in the study, we utilized a partial least squares discriminant analysis (PLS-DA). The PLS-DA model using all four conditions exhibited a moderate degree of predictive accuracy, but the low Q2 values indicate that the model may be overfitted to the data given the small sample size.

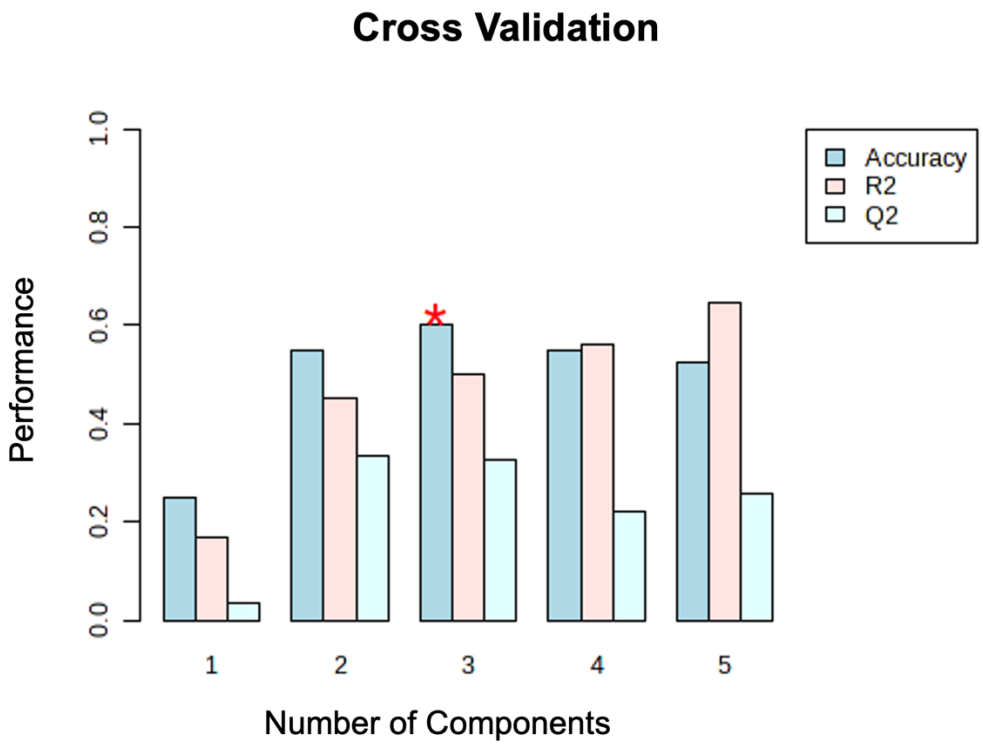


Figure 1: PLS-DA Cross Validation

The scores plot indicates that there is variation in metabolite expression between the two varieties of soybean, as the metabolic profiles are more similar between the healthy/dying cohorts of a variety than they are to the respective cohort in the opposite variety. This may pose a challenge for future data analysis, as it may cut the sample size in half.

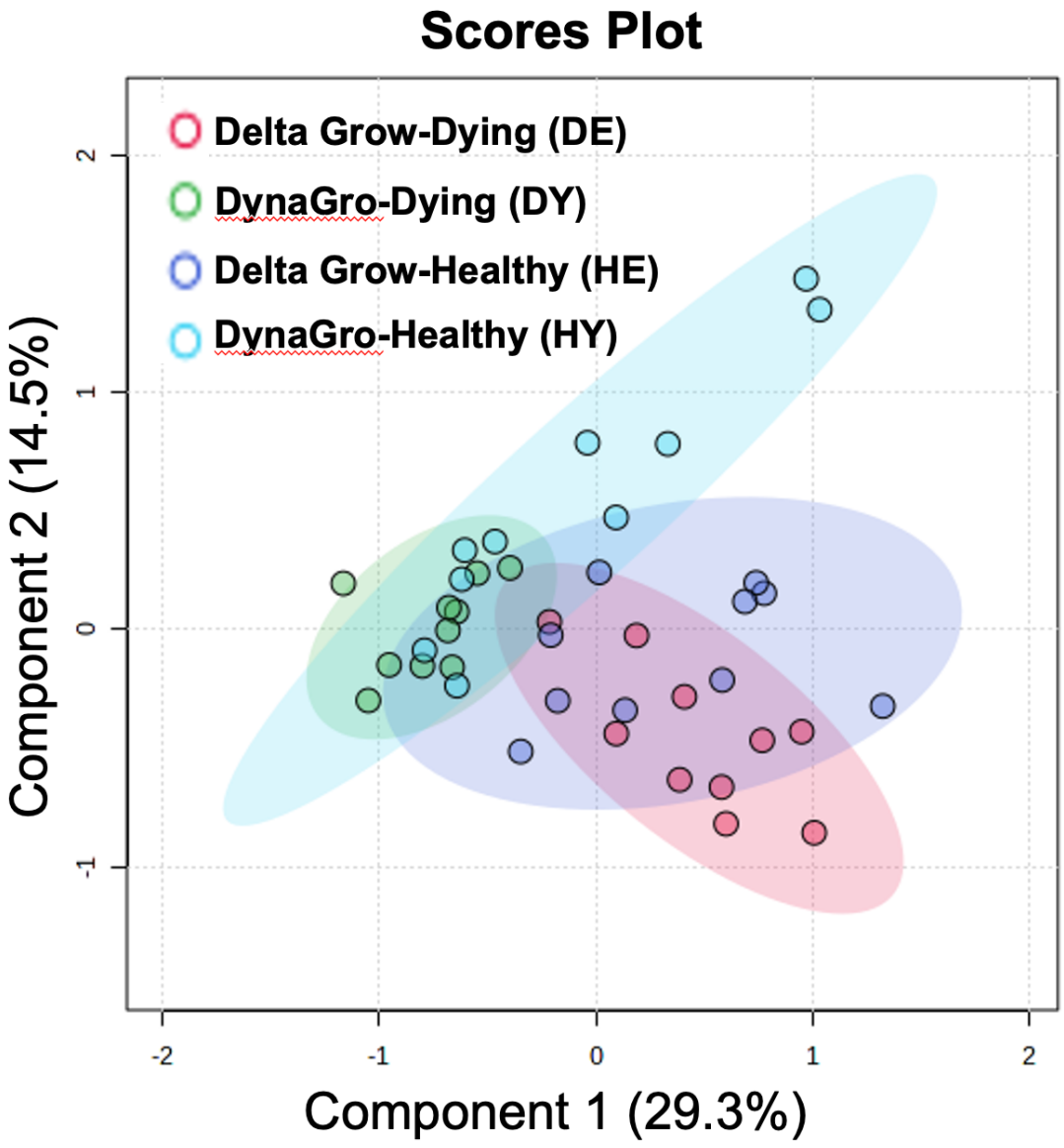


Figure 2: Scores Plot

Of the two varieties, only Dyna-Gro S48XF35 exhibited statistically significant differences in metabolite concentrations between healthy and dying samples.

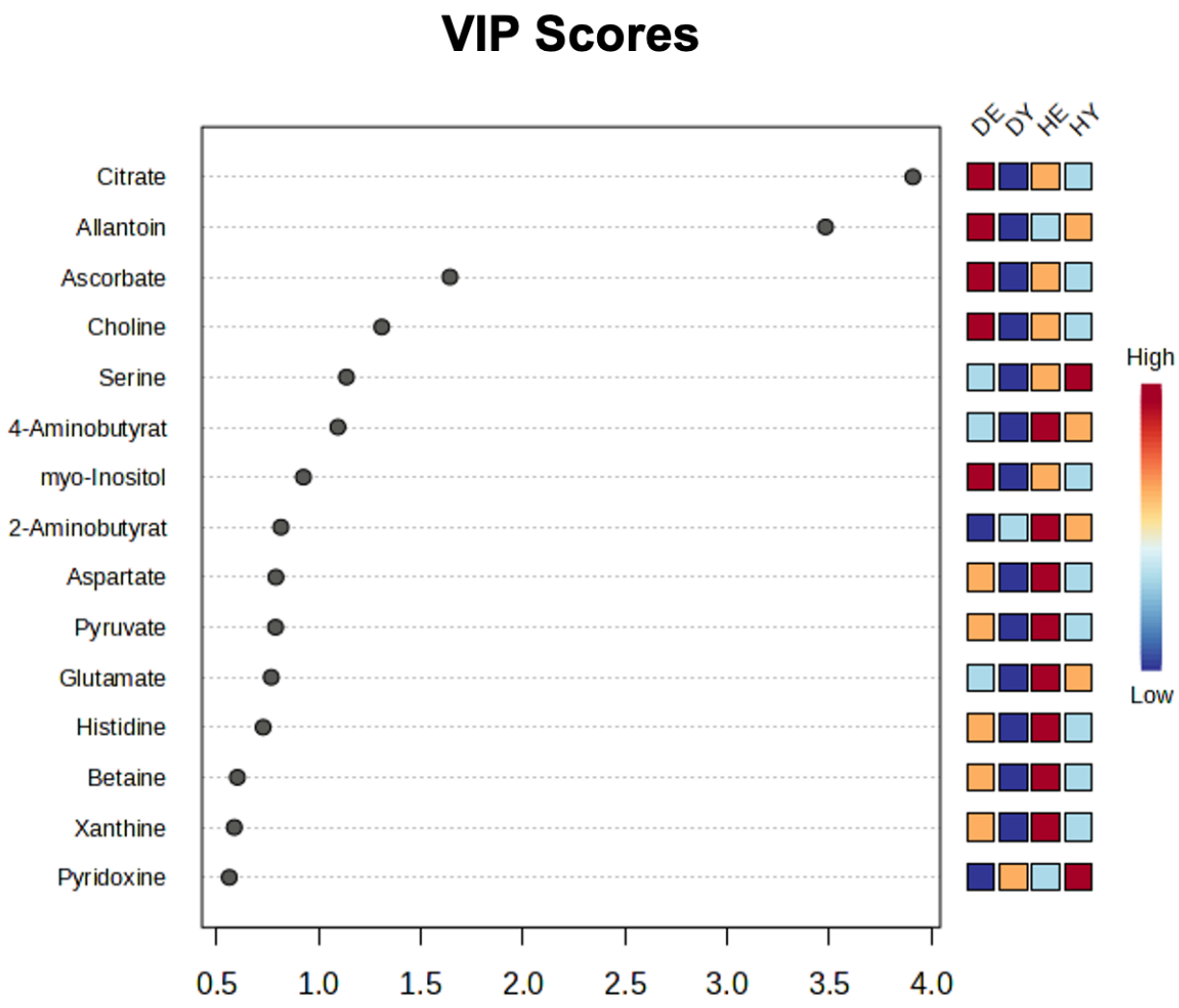
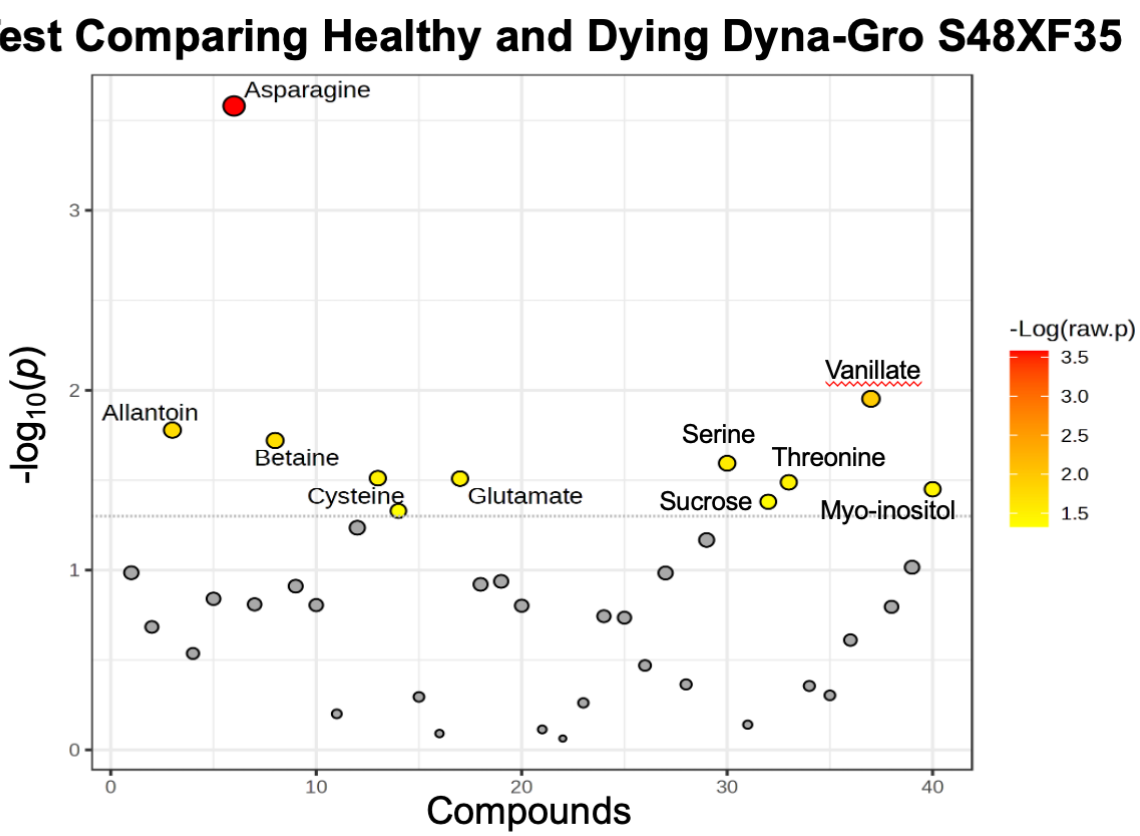


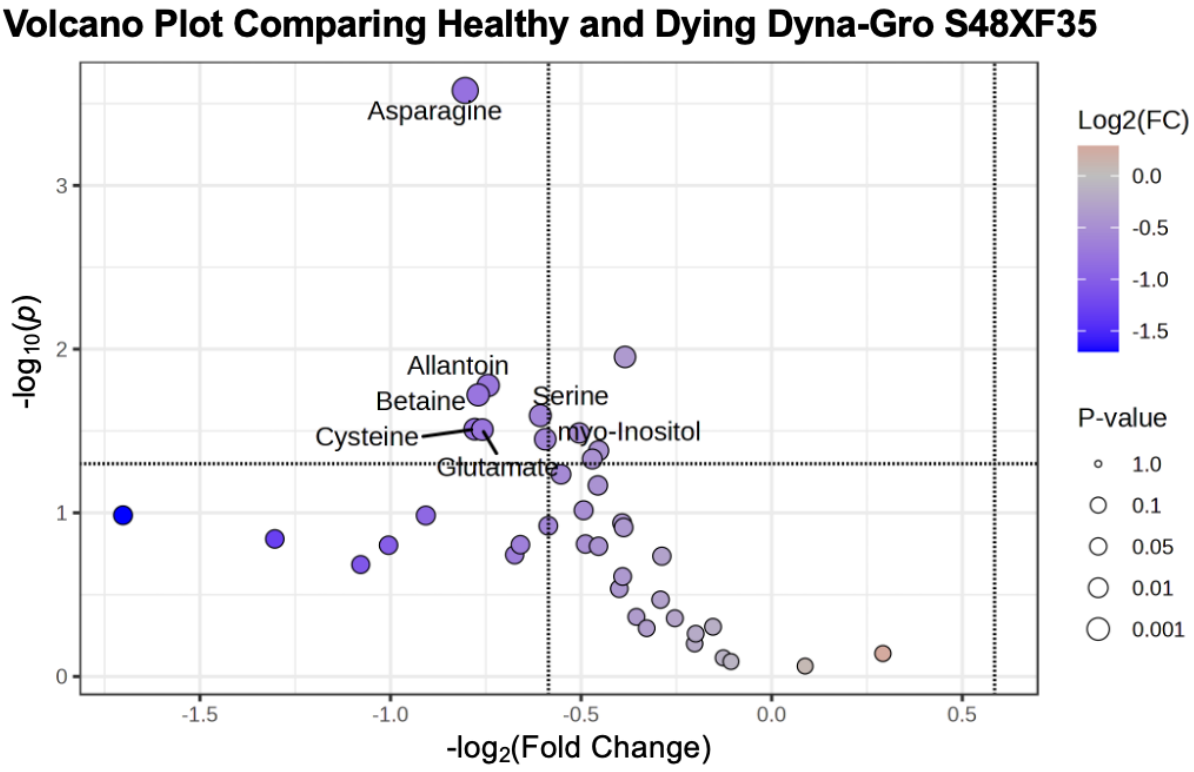
Figure 3: VIP Scores

Asparagine was very significantly different ($p < .001$) between the healthy/dying plants and was also statistically significantly different in the MP greenhouse trials previously conducted in the Baird Lab. Serine ($p = .025$) was also found to vary significantly between MP positive and MP negative samples.



	t Statistic	*p* Value	-log$_{10}$(*p*)	FDR
Asparagine	-4.524	< .001	3.581	0.011
Vanillate	-2.827	0.011	1.952	0.158
Allantoin	-2.639	0.017	1.778	0.158
Betaine	-2.576	0.019	1.721	0.158
Serine	-2.436	0.025	1.594	0.158
Cysteine	-2.343	0.031	1.511	0.158
Glutamate	-2.340	0.031	1.508	0.158
Threonine	-2.317	0.033	1.488	0.158
myo-Inositol	-2.273	0.036	1.450	0.158
Sucrose	-2.193	0.042	1.380	0.167
Cystine	-2.134	0.047	1.329	0.171

Figure 4: T-Test Comparing Healthy and Dying Dyna-Grow S48XF35



	Fold Change	$\log_2$(Fold Change)	*p* value	$-\log_{10}(p)$
Asparagine	0.573	-0.804	< .001	3.581
Allantoin	0.597	-0.744	0.017	1.778
Betaine	0.586	-0.770	0.019	1.721
Serine	0.657	-0.607	0.025	1.594
Cysteine	0.583	-0.779	0.031	1.511
Glutamate	0.591	-0.759	0.031	1.508
myo-Inositol	0.663	-0.594	0.036	1.450

Figure 5: Volcano Plot Comparing Healthy and Dying Dyna-Gro S48XF35

Overall, there are three main findings in this data. The first is found in the PLS-DA model. This model exhibits a predictive nature to how the soybeans will react to the fungal disease. However, with the low Q2 value, it suggests potential overfitting due to the small sample size of the study and may could become more reliable in predicting the reaction of the soybeans with a larger soybean sample size. The second finding can be found in the metabolic differences. There were more pronounced metabolites in one variety compared to the other regardless of health status. This could complicate further analysis and make this process of

understanding the reaction of the soybean to Mp could limit future comparisons such as this one in this study. Thirdly, metabolic changes between healthy and dying plants. It is seen that only Dyna-Gro S48XF35 showed a significant metabolic change between the healthy version of the plant and the dying plant. The metabolites that were strongly associated with plant health are asparagine (consistent with previous study in greenhouse) and serine (varied by Mp status). These findings present the opportunity for these metabolites to be key metabolic indicators of resistant plants to Mp and this information could be utilized in future breeding efforts to create a Mp resistant soybean.

Current and Further Research:

Breeding for resistance is difficult for fungal diseases like charcoal rot disease, which are controlled by multiple genes. To support breeding efforts for Mp resistance, phenotypic biomarkers of host metabolites, associated with different or related metabolic pathways, could be used to inform breeding targets, similar to as demonstrated for some human diseases. Metabolomics research using the nuclear magnetic resonance (NMR) platform can provide metabolic profiles for plant disease or from other stress factors. NMR spectroscopy can be used for large scale collection of informative metabolomic data. These metabolic fingerprints could provide viable biomarkers between healthy and diseased plants and potentially identify individual or groups of metabolites during the disease (toxin) cycle. To further the findings of this research, we could build upon the potential of certain metabolites having acute resistance to this disease. These plants can be planted and a study determining their yield could determine whether or not Mp affects these slightly resistant plants more severely, less severely, or the same as non-resistant soybean plants.

Fellowship Takeaways and Acknowledgements:

This time in the lab has been invaluable to my outlook on the research process and how to approach working in a team on an academic project. My previous times in the lab have been one on one whereas this one has been part of a larger team with many moving parts. Having the chance to approach this project and experience a new perspective on the research process has allowed me to be shaped into the researcher I am today, and this would not have been possible without the funding of the honors college. The funding allowed me the chance to buy NMR tubes last semester to conduct this research in the NMR lab in the Hand Building. The funding this semester also allowed me to get paid for my efforts in the lab so that I was not strained financially getting hands-on experience in the lab. Presenting my poster at the undergraduate research symposium allowed me to better my skills in academic communication. Telling people about the research with only a poster to reference was an experience to help me to improve my abilities to be a better communicator for people outside and inside my field of study. Taking questions from the judges helped me think critically about my research and to put all my knowledge on the topic to use. I have immense gratitude I have for the honors college to help fund this endeavor and allow me to get a chance to work in a R1 lab with a group of scientists that have taught me so much about the scientific process.

Name: Lily Langstaff

Faculty Advisor: Dr. Beth Baker

Project Title: *DR-4429: Social Perceptions and Narratives of the 2019 Mississippi Delta Flood*

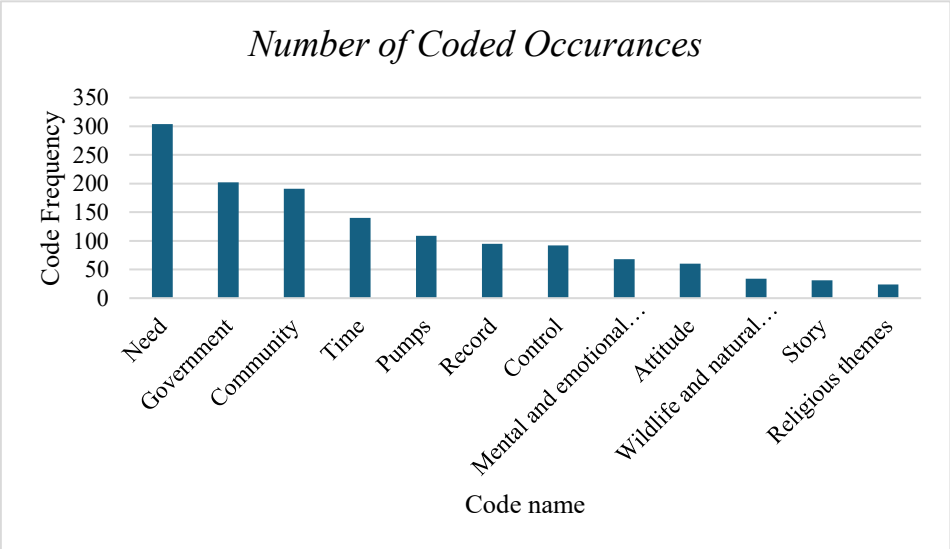
Introduction: During the 2024-2025 academic year, for my Shackouls Honors Thesis, I completed a thematic mixed-methods media analysis of the 2019 Mississippi Delta backwater flood. My goal was to understand public narratives, stories and perceptions surrounding the flood to properly evaluate both community level impact and response (Holzhausen & Grecksh 2021).

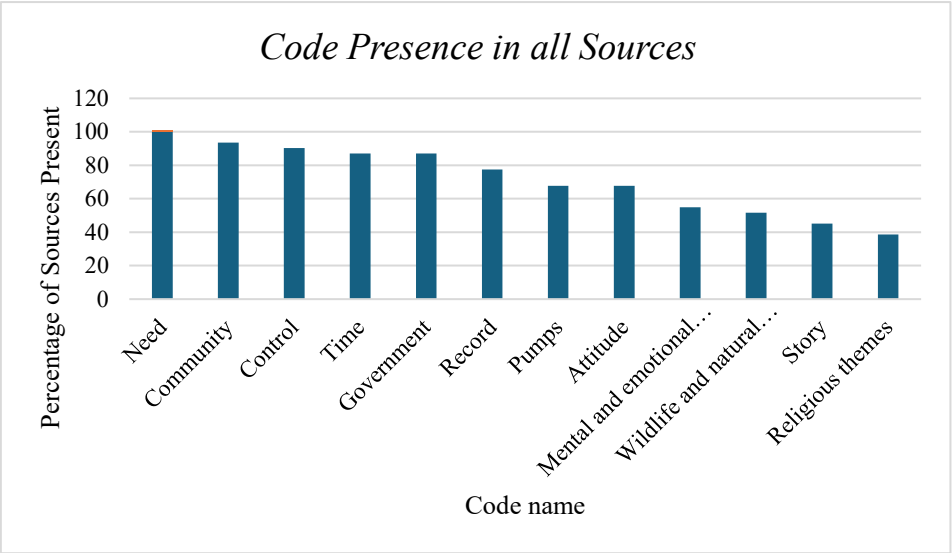
I aimed to develop a series of policy recommendations to more equitably and efficiently serve the Delta backwater community. The final recommendations focused on temporal marginalization (or marginalization caused by long waiting periods), public engagement in decision-making processes, community resilience, and improvements in insurance and infrastructure to protect the backwater community. It is a historically underserved and predominately African American area, with disproportionately high rates of poverty and disability as well as a lower rate of education (U.S. Census). The significance of this research lies in the community need for improvements in policy and management, particularly through infrastructure, captured in the specific recommendations developed.

Methods: I reached this goal by analyzing journalistic media across global, national, state, and local level digital newspapers. First, I completed the source retrieval by searching '2019,' 'Mississippi,' 'Backwater,' 'Flood' in Google and using the 'News' tab, in order to find the most publicly accessible content. If a source had more than three articles under the search terms, the most recent three were utilized. Other than this criteria, I analyzed all of the articles that resulted from the search. The second step was to read each of the articles to familiarize myself with the overall content. Then, I completed a primary stage of coding in

the MAXQDA coding software by assigning themes relevant to and repeating within the text as specific codes (VERBI Software, 2024). Once each article had been thematically coded, 'thematic saturation' was reached, where the final list of all codes was determined. With this list, I completed a secondary stage of coding to unsure thorough thematic content analysis (Braun and Clarke 2006). Finally, I utilized the coded dataset to conduct a quantitative analysis of code frequency, distribution and correlation. These statistics allowed me to evaluate the meaning and significance of each code as well as relationships between codes.

Results: 31 texts were analyzed with MAXQDA, and 13 main codes and 2,421 total coded segments emerged. The primary journalistic style was narrative storytelling, where figures and personal stories and anecdotes were highlighted. The code names alongside number of occurrences as well as percentage of documents included within were as follows:





The most prominent themes were need, community, government, time, and control.

Need: Need had the largest presence within all sources, with the highest overall frequency (304), and appearances throughout all 31 documents. “Need” is viewed in this analysis context as individuals or groups impacted by the flooding and having needs that are not met, and demonstrating other circumstances of need or vulnerability, such as being elderly or disabled.

Community: Community was also a dominant theme throughout the analysis, with clear flood impacts demonstrated throughout different aspects of the local community through 191 coded segments across 94% of documents, making it the third highest in overall frequency and the second highest in number of documents included in. This theme captures residential impacts and quotes demonstrating how flooding shaped local life.

Government: Varying sentiments surrounding government were present throughout roughly 87% of the documents. Ideas surrounding government were coded 202 times, making it the second highest in terms of overall frequency. Beliefs of “government responsibility” was a common subcode, along with sentiments “distrust or lack of reliance,” instances of residents being “politically vocal or engaged, participating in meetings,” and mentions of “MS officials in support of pumps.” “Insurance or assistance” was also a popular subcode, with a range of

secondary subcodes including “insufficient or not covered” (the most abundant), “unsure about sufficiency” (the second most abundant), and “sufficient or not needed or used” (the least abundant).

Time: Time was a dominant theme throughout the analysis, with 140 coded segments across roughly 87% of documents. It had several subcodes including “months,” “years,” “slow – never-ending duration,” “fast – flooding and impacts,” and “other temporal displacement.” Many coded segments described lengthy waiting periods, such as months of inundated housing or years without proper governmental support.

Control: There were 92 instances of perceived control across roughly 90% of documents, making it the third highest in terms of number of documents it appears in. Strong sentiments of residents not being in control of the cause of their situation (cause control) were present. In addition, sentiments or stories captured residents putting effort into controlling their situation (situation control), such as reinforcing sandbag barriers, though these efforts were oftentimes not enough to prevent flooding.

The thematic analysis, through the utilization of both qualitative media evaluation and the paired statistical analysis, suggested that there was a strong need for assistance, a strong sentiment of community, a strong distrust in government despite a sense of responsibility associated with government, a strong time-related impact (temporal displacement), and an effort to be in control of the situation despite a lack of control of its cause. I found that, in the specific case of the 2019 Delta backwater flood, temporal displacement led to community action and involvement, which to a high extent involved political participation and activism. This understanding of the more specific impact and overall context helped me develop each of the policy recommendations included in the introduction of this report.

Future Directions: My recommendations for future research included a deeper and more specific analysis of any of the themes identified throughout the project for more focused

policy recommendations, as well as a broader analysis of other forms and time frames of media, such as songs documenting the Flood of 1927. For future research, I also recommend combining thematic and statistical, or qualitative and quantitative, analysis methods. This allowed me to produce significant and methodological research with a specific context or narrative to better understand and explain it.

Research Experience: I conducted this research independently, from conducting a literature review and developing the methodology to sourcing, conducting, and analyzing the media. However, I did work under the excellent mentorship of Dr. Beth Baker, who was consistently available for feedback and revisions. The research funding served mainly as hourly wages that allowed me to execute this research through a semester abroad at the University of Oxford and allowed me to utilize the MAXQDA software without personal expense. This thesis is a culmination of my academic passion and drive, both of which I have developed further through the experience. It is a project that I described in my graduate school applications, and I hope to conduct similar research as I begin a Master of Public Policy in the fall of this year.

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