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Founded in 1873, the Minnesota Academy of Science (MAS) is a statewide 501(c)3 organization committed to advancing science, technology, engineering, and math (STEM) in Minnesota by connecting Minnesotans of all backgrounds with resources and opportunities to engage in STEM learning, research, and communication—and to recognize excellence in these areas.

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JOURNAL OF ABSTRACTS OF THE MINNESOTA ACADEMY OF SCIENCE

Vol. 83 Spring 2021

TABLE OF CONTENTS

The abstracts included in this publication describe research presented at the Minnesota Academy of Science Annual Meeting / Winchell Undergraduate Research Symposium held virtually on April 24, 2021.

BIOCHEMISTRY

DETECTION OF ATRAZINE METABOLITES IN BLOOD SERUM AND LIVER OF MICE EXPOSED VIA THEIR WATERS TO 0, 3, AND 30 PPB ATRAZINE

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Atrazine, which is an herbicide used on crops throughout the Midwest, is present in drinking water due to its ability to be carried in runoff from fields. While levels of less than 3 ppb in drinking water are deemed as safe by the USDA, atrazine is often found at much higher levels in nature due to the widespread use of this chemical in fields. Recent studies have identified that atrazine decreases Glutathione-S-transferase (GST) gene hepatic expression when mice are exposed to atrazine in their drinking water. This study investigated if there was a build-up of atrazine metabolites in the serum and liver of exposed mice and correlated the species of metabolite amount to the effect it has on gene expression of GST.

SERTRALINE BINDING TO THE S1S2 DOMAIN OF IONOTROPIC GLUTAMATE RECEPTOR GLUN1 SUBUNIT

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The molecular mechanism of depression is currently not fully understood. The focus of this research rests in where the antidepressant, sertraline, binds to the S1S2 domain of the NMDA ionotropic glutamate receptor GluN1 subunit. Computational analysis was utilized to acquire binding information. POCASA (Pocket Cavity Search Algorithm) identified five potential pockets for sertraline binding, and Swissdock provided data identifying one of them as the predominant pocket for sertraline binding. Analysis of this binding showed the presence of hydrogen bonds between the nitrogen of sertraline and asparagine 48 and histidine 12 in the S1S2 domain. Further analysis revealed electrostatic interactions between the chlorine of sertraline and lysine 20, lysine 91, and histidine 12 in the S1S2 domain. To probe this binding, the histidine 12 of the receptor was mutated to phenylalanine. Results of this computational mutational analysis will be presented. With cases of depression on the rise as the COVID pandemic continues to shake our communities, understanding the mechanism of depression will provide clarity in the effectiveness of treatment for millions of Americans.

IS CYCLIC ELECTRON TRANSPORT IMPORTANT FOR PHOTOPROTECTION DURING WINTER STRESS IN *Pinus strobus* AND *Pinea glauca*?

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Winter stress caused by extreme cold causes evergreen trees to reduce their rate of carbon fixation, but they are still absorbing the same amount of sunlight, causing them to have excess energy that they need to cope with. We monitored chlorophyll fluorescence and protein levels during recovery from winter stress in two evergreens: Eastern white pine (*Pinus strobus*) and white spruce (*Pinea glauca*). We predicted that there would be increases in cyclic electron transport during winter to help cope with excess light energy. To test this, we monitored levels of key photosynthetic proteins and their phosphorylation status during spring recovery. We found that white spruce recovered more quickly in the spring and maintained higher levels of cytochrome f and a photosystem I reaction center protein than white pine, which supports a role for increased cyclic electron transport. Also, we found that white spruce showed more dramatic changes in phosphorylation of key photosynthetic proteins during low

temperature periods. Taken together the data suggest that white spruce may increase its use of cyclic electron transport during winter, more so than white pine, allowing for a faster recovery in the spring.

ALTERNATIVE CARDIAC PACEMAKER MECHANISM

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The heart's conduction system has been a mechanistic wonder for scientific research. Studying the makeup of the pacemaker cells has led to multiple advancements in technology, and innovative ways to treat patients with arrhythmias. Yet many unanswered questions remain. One example is how does the atrioventricular node know when to switch between being a delayer to a secondary pacemaker? This research focuses on answering that question through the meta-analysis of scientific literature on cardiac rhythm studies. The dual purpose of the atrioventricular node is not due to one factor, but multiple, consisting of early development, anatomical structure, connexin proteins, and unique ion channels. During early development, Tbx3 coordinates the repression and expression of SAN-specific genes and the atrioventricular canal formation, directly impacting the formation of the atrioventricular node and its divisions, the compact node (CN) and the lower nodal bundle (LNB). Additionally, Tbx3 coordinates the specific gap junction connexin proteins Cx43 and Cx40 depending on AV node location. CN expresses Cx40 and represses Cx43, the opposite of which is seen in the LNB where the AV node resides. This connexin ratio tunes AV node delay. The self-pulsation of pacemaker cells is due to specific channels encoded by the hyperpolarization-activated cyclic nucleotide-gated gene family (HCN), which are dually activated by hyperpolarization and cAMP, therefore showing that our comprehension of this system is not complete. That being the case, more research is critical to help companies like Medtronic, Boston Scientific, and Abbott Laboratories find new ways to improve pacemakers.

THE ROLE OF FFAR4 IN NAFLD/NASH

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Nonalcoholic fatty liver disease (NAFLD) is an emerging healthcare problem worldwide, and the disease parallels the increase in obesity due to the drastic change in lifestyle habits during the last century. Many patients with NAFLD will develop a more inflammatory subtype, nonalcoholic steatohepatitis (NASH), which could then progress to advanced liver disease, cirrhosis, and hepatocellular carcinoma. Different characteristics of NASH could lead to the progression of more advanced liver disease, but our study focused on the function of macrophages, whose secretion of cytokines has been found to promote disease progression. There are two subsets of liver macrophages: Kupffer cells, which stay in the liver and locally proliferate and self-sustain, and infiltrating monocytes, which derive from bone marrow-resident haematopoietic stem cells. In different stages of liver disease, Kupffer cells and freshly recruited monocyte-derived macrophages are both involved in the regulation of inflammation, fibrogenesis, and fibrolysis. In metabolic diseases such as obesity, NAFLD, and NASH, increased lipid turnover in expanding adipose tissue leads to elevated serum free fatty acids (FFAs), and an important correlation exists between obesity and higher levels of the saturated fatty acid palmitate. Palmitate is thought to interact with Toll-like Receptor 4 on the surface of cells and initiate pro-inflammatory programs. However, other fatty acids, like omega-3 fatty acids, can bind to the cell-surface FFA receptor FFAR4 and have anti-inflammatory functions. The goal of our project was to determine if macrophage FFAR4 plays a role in the progression of NAFLD/NASH.

EFFECTS OF THE P126E MUTATION ON MALATE DEHYDGROGENASE'S SPECIFIC ACTIVITY AND TURNOVER NUMBER

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Malate dehydrogenase catalyzes the reversible conversion of oxaloacetate to malate while simultaneously oxidizing NADH to NAD+. This reaction is of interest because it plays a key role in the tricarboxylic acid cycle, gluconeogenesis and amino acid metabolism. A flexible loop (P119-N137) within MDH's active site contains two of three arginines whose positive charge interacts with the negatively charged 4-carbon dicarboxylic acid substrate to coordinate its orientation for catalysis. The aim of this study was to investigate how a loop mutation at position 126 (P126E) affects malate dehydrogenase's specific activity and turnover number. We hypothesized that changing proline to glutamate reduces the catalytic loop's flexibility, making it less able to orient and stabilize the substrate in the active site. As a consequence, the P126E mutant is expected to have lower specific activity and turnover number than the wild type malate dehydrogenase. To address this hypothesis, we used a watermelon glyoxysomal MDH (WMgMDH) as a model system and constructed a P126E mutation in the loop using Quickchange mutagenesis. The wild type and mutant enzymes were overexpressed in *Escherichia coli* and purified using Ni-NTA affinity chromatography. Enzyme activity was monitored by measuring absorbance at 340 nm to follow the oxidation of NADH to NAD+. The results showed that the mutant enzyme, P126E, had a 10-fold lower specific activity and turnover number than the wild type enzyme. These findings suggest that the proline residue at position 126 in the MDH loop plays a role in substrate binding and catalysis.

DESIGNING A 3D PHYSICAL MODEL OF HIV PROTEASE TO EXAMINE BIOCHEMICAL INTERACTIONS AND DRUG RESISTANCE TO TIPRANAVIR AND DARUNAVIR

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HIV kills approximately one million people worldwide every year. Despite advances in antiretroviral therapies, drug resistance remains a major treatment challenge. HIV protease aids viral replication by facilitating the peptide bond hydrolysis reactions required for the formation of mature virions. To target the active site and substrate envelope of HIV protease, various inhibitors have been developed. While these drugs are effective at inactivating the enzyme, mutations at the active site result in HIV resistance strains that are insensitive to protease inhibitors. The goal of this project was to build 3D printed models of HIV protease to demonstrate the mechanisms of action of two inhibitors, Tipranavir and Darunavir, as well as the mutations that contribute to drug resistance. To identify conserved amino acids and structural features important in the catalytic mechanism of HIV proteases, database searches and sequence alignments were performed. The Protein Data Bank Files 6DIF, 6DGX, 6OPS, and 6DH6 were analyzed to learn more about the protein structure and its interactions with Tipranavir and Darunavir. The structure files were imported into Jmol and converted into a format suitable for 3D printing. The physical models constructed illustrate the biochemical interactions between the enzyme and the inhibitors. Additionally, the models highlight important amino acids like Asp25, Ile50, Val82, and Val84, which are important for catalytic activity and the development of drug resistance. A Jmol tutorial was designed to complement the 3D models and assess students' learning of the structure and function of HIV protease.

ANALYSIS OF CIRCADIAN PLASMA CORTISOL LEVELS IN ATRAZINE EXPOSED MICE

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Cortisol is an adrenal steroid released by the HPA axis as a stress response, which has been shown to be regulated by sex hormones. Atrazine is an endocrine disrupting herbicide. To test the effects of atrazine exposure on cortisol levels, *Mus musculus* were exposed to 0 ppb, 3 ppb, or 30 ppb for a period of four weeks. Because cortisol has been shown to have circadian expression, blood plasma was harvested at six time points and analyzed for shifts. It was found that exposure at both 3 ppb and 30 ppb caused a shift in the circadian expression of cortisol.

CELLULAR AND MOLECULAR BIOLOGY

FROM SOIL TO *Pseudomonas*: THE JOURNEY OF A BACTERIUM IN THE TINY EARTH RESEARCH STUDY

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Antibiotic resistance is a growing crisis throughout the world. Screening bacterial isolates from soil for antibiotic production is a way to combat this issue. There are over 1011 bacteria per gram soil and these bacteria produce secondary metabolites that inhibit the growth of other microbes. Additionally, soil bacteria differ from area to area, so there is much untapped potential. We are collaborating with the Tiny Earth project, which is a student sourcing endeavor to test bacterial soil isolates to discover new antibiotics from all around the world. A soil sample was collected in Andover, Minnesota and to isolate bacteria it was diluted and plated on a PDA agar plate. Twenty-four isolates were tested for inhibition against safe relatives. Safe relatives are non-pathogenic organisms that are safe to use in lab but have similar characteristics to the more deadly ESKAPE pathogens known as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. It was determined that two of the isolates from my soil sample inhibited the safe relative *Pseudomonas putida*. To identify one soil isolate, I performed a series of tests on the isolate such as gram staining, specialized media testing, biochemical characterization, and 16s rRNA sequence analysis. These tests revealed that the isolate was of the genus *Pseudomonas*. Further tests are needed such as structural determination of the inhibitory compound and evaluating activity against eukaryotes to know if the isolate produces a novel and potentially useful therapeutic antibiotic.

ANALYZING THE ANTIBIOTIC PRODUCTION AND GENOME OF A *Pseudomonas* STRAIN FROM THE SOIL

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The rise in antibiotic use over the last century has been integral in treating bacterial infections but has also contributed to the proliferation of many antibiotic-resistant strains of bacteria. Discovery of novel antibiotics is expensive and time-consuming with very little payoff for pharmaceutical companies. The Tiny Earth Program student-sources antibiotic discovery through isolating and analyzing antibiotic-producing bacteria from the soil. Bacteria are abundant in soil, and about 75% of the antibiotics in use today originated from soil bacteria. In my work, twenty-four strains of bacteria were isolated from a soil sample originating in Arden Hills, Minnesota and were tested for their ability to inhibit the growth of three different safe relatives of common superbugs. One soil isolate produced zones of inhibition against all three, suggesting it may produce a broad-spectrum antibiotic. The

16s rRNA gene of this species was amplified using PCR and sequenced. The results were analyzed using a nucleotide BLAST search, and it was identified as *Pseudomonas*. The full genome was later sequenced through the Tiny Earth Chemistry Hub, and the species was determined to be *Pseudomonas migulae*. Using antiSMASH analysis, eight genomic regions potentially responsible for the production of secondary metabolites were identified. Three show similarities to known antimicrobials, and three appear to be novel. Sensitivity of eukaryotic cells to the isolate as well as chemical extraction to purify the active compound were performed to investigate its potential for use as a novel therapeutic.

DETECTION OF AN ANTIBIOTIC PRODUCING *Pseudomonas* BACTERIUM FROM SOIL

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The need to identify new antibiotics is critical as there is an increasing issue of antibiotic resistance among bacteria. Tiny Earth is an undergraduate research program that is assisting in the discovery of new antibiotics. The purpose of this research was to determine if a bacterium that produced antibiotics could be found from a soil sample. Soil contains numerous organisms and bacteria which makes it fertile ground for research. A sample of soil was obtained from the University of Northwestern – St. Paul lawn and bacteria were isolated by growing on tryptic soy agar plates. Twenty-four isolates were screened for antibiotic production by testing them against ESKAPE pathogen safe relatives to harmful bacteria. One antibiotic producer showed inhibition to both *Pseudomonas putida* and *Staphylococcus epidermidis* and another antibiotic producer showed inhibition to just *Staphylococcus epidermidis*. The former was selected to characterize. To identify the isolate, the 16S ribosomal RNA gene was amplified using polymerase chain reaction and sequenced. BLAST analysis revealed it was in the genus *Pseudomonas*, a common bacterium in the environment. Biochemical testing and gram staining were consistent with this finding as the isolate was a gram-negative bacterium. Gaining knowledge about these producers can give insight for future research as we seek to develop new medications to treat bacterial infections.

DISCOVERY OF AN ANTIBIOTIC PRODUCING BACTERIUM

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Today, we are in a crisis in which bacteria is becoming resistant to antibiotics. Many physicians are prescribing antibiotics to people who do not necessarily need that antibiotic or prescribing too much of it. Also, many people do not fully finish their medication which causes that specific bacteria to become resistant to the antibiotics. This is a problem because as more antibiotics are being misused, the more resistance is growing among them, making it more difficult to treat people in need. The purpose of this semester-long research project, done in collaboration with the Tiny Earth network, was to discover a new antibiotic producing bacterium from soil. Studying bacteria from the soil is fruitful because of the abundance of bacteria in soil and their metabolic potential. A soil sample was obtained from the campus of University of Northwestern, St.Paul, MN. A bacterium grown from this sample inhibited *Bacillus subtilis*. Sequencing the 16s rRNA gene, revealed that the isolate was of the genus *Pseudomonas*. A Gram stain and biochemical tests were performed to characterize the isolate. Results were consistent with it being *Pseudomonas*. Further work must be done to determine if the bacterium produces novel antibiotic.

THE EFFECT OF TBX2 OR EPIREGULIN ON THE ACTIVATION OF EGFR, HER2, HER3, AND HER4 IN THE MCF10A CELL LINE

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Breast cancer is one of the most prevalent forms of cancer in the United States and affects more than three million women worldwide. Almost half of those diagnosed with breast cancer have been associated with an overexpression of TBX2. Over expression of TBX2 has demonstrated to bypass senescence and create tumorigenic cells. TBX2 overexpression has also been linked to increased levels of epiregulin. The link of Epiregulin to Epidermal Growth Factor Receptors Her1, Her2, Her3, and Her4 has been supported but not fully proven. Studies in the past have shown epiregulin binding to and activating the EGFR family EGFR, Her2 Her3, and Her4. Epiregulin has also been shown to bind to but not fully activate Her 2. The objective of this study was to determine, in the MCF10A human mammary tissue cell line, if TBX2 overexpression through transfection and/ or epiregulin over expression leads to the activation of EGFR family. Activation of the EGFR family leads to cellular responses such as activation of the Ras-MAP kinase pathway which triggers cell proliferation, growth and survival. Presence and activation of the EGFR family was determined through immunoblots. Findings of this study aim to gain a greater understanding of how and why drugs, such as Herceptin, a Her2 inhibitor, work and could lead to better treatments that could target more protein receptors.

INVESTIGATION OF ANTIBIOTIC-PRODUCING SOIL BACTERIA

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As antibiotic resistance becomes an increasingly serious problem, it is necessary to develop alternatives and new antibiotics. Tiny Earth is attacking this problem by utilizing the skills of students to engage in a widespread effort of antibiotic discovery. We sampled soil, which tends to be rich in symbiotic and metabolically active bacteria, in an attempt to find natural antibiotic producers. We hypothesized that we would observe antibiotic production against one or more of the ESKAPE pathogen safe relatives of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). We sampled soil from St. Paul, Minnesota, which was then diluted and plated onto LB plates. Twenty-four bacterial isolates were tested against ESKAPE pathogen safe relatives and viewed for zones of inhibition. Among our isolates, one appeared to be an antibiotic producer. To identify the genus of this soil isolate, we performed gram staining, 16s rRNA sequencing and biochemical and specialized media testing. The isolate was shown to be an *Arthrobacter*. Further chemical analysis of the antibiotic being produced by this isolate may reveal biologically and pharmaceutically relevant information that could augment our understanding of antibiotics, or at best, provide a basis on which to produce new pharmaceuticals.

EXAMINATION OF THE EXPRESSION OF ETV1 DURING LUNG DEVELOPMENT IN *Xenopus laevis* IN RESPONSE TO FGF SIGNALING

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All air-breathing animals require lungs for survival. Aberrant development of the lungs can induce considerable disease and complications, especially in pre-term babies. Gaining insight and further understanding of lung development should lead to better treatment and prevention of these diseases and complications. While many signaling pathways are integral to the development of the lungs, the fibroblast growth factor (FGF) pathway has been shown to play a critical role in lung morphogenesis. It has been shown that reduced lung development in *Xenopus laevis* is a consequence of inhibiting FGF signaling in, while increased FGF signaling was correlated

with an increase of lumen size in lung epithelium. It is known that etv1 is expressed in developing *Xenopus* lungs and in this study we demonstrated that etv1 is expressed in both the endoderm and mesoderm. Additionally, we wanted to investigate if etv1 lung expression responds to changes in FGF signaling through microinjections and small molecule inhibitors. We present that the downregulation of FGF signaling is proportionally linked to the expression of etv1. Additionally, the effects of ectopic expression of nkx2.1 on etv1 expression were investigated. We found that the overexpression of nkx2.1 is correlated with the overexpression of etv1 and confirm the correlation with the overexpression of sftpc.

WARTBURG COLLEGE TINY EARTH PILOT PROGRAM: SOIL BACTERIA AND ANTIBIOTIC DISCOVERY

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Some types of soil bacteria are known to produce antibiotics in their natural environment to increase their competitive advantage over other bacterial species. To discover antibiotic-producers in soil samples, we are using resources from the Tiny Earth research network. Our long-term goal is to implement components of the Tiny Earth program into future Microbiology courses at Wartburg College. The Tiny Earth program provides students with background information, methods, and resources to culture, identify, and test soil bacteria for antibiotic production. The short-term goal of this research is to explore a sample of soil bacteria using the Tiny Earth resources and identify an antibiotic-producing bacterial species. To culture and identify antibiotic-producing soil bacteria, we isolated and cultured bacteria from a compost sample onto Trypticase Soy Agar (TSA) and Nutrient Agar (NA) plates, using a serial dilution of the sample to attain individual colonies. Next, we identified 23 individual, unique bacterial colonies, described the morphology of these colonies, and patched them onto a master plate. We tested the 23 sample bacteria for antibiotic production by culturing them on plates with *Escherichia coli* and *Staphylococcus epidermidis*, respectively. One sample bacteria colony created a halo-like zone of inhibition around itself, a sign of antibiotic production. Our next steps are to sequence and identify this bacteria species, test it further for antibiotic production and identify the antibiotic it produces. To meet our long-term goal, we share dour findings with the current Microbiology course at Wartburg College and implemented our methods into future Microbiology lab activities.

HOW DOES NLRP2 CONTROL FETAL DEVELOPMENT?

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NLRP2 is a member of the NOD-Like receptors family of proteins, which play an important role in innate immune responses. Some NOD-like receptors, like NLRP3, form a multi-molecular protein complex known as the inflammasome that activates caspase-1, which then activates inflammatory cytokines like IL-1β. Other NLRs, such as NLRP 12, may also function as regulators of inflammation. To investigate other proteins and pathways that NLRP2 may play a role in, we designed a Yeast 2-Hybrid assay with a human cDNA library to screen for any human proteins that interact with NLRP2. We identified a protein interaction between NLRP2 and PA2G4(Human Proliferation-associated protein 2G4), a protein that plays a role in development. Through other projects, we have found that PA2G4 interacts with NLRP7 which is responsible for hydatidiform molar(HM) pregnancies. Once the interactions are confirmed, we plan to expand our research to identify the involvement of NLRP2 in molar pregnancy.

THE IDENTIFICATION OF AN ANTIBIOTIC-PRODUCING ISOLATE FROM SOIL

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The issue of antibiotic resistance has been an increasing concern across the world. Antibiotics are regularly showing ineffectiveness against ESKAPE pathogens, and discovery of new antibiotics has proven to be a tedious task. The purpose of our research, done in affiliation with Tiny Earth, was to identify an antibiotic-producing bacterium from soil. Soil was used for the purpose of this research as it naturally contains bacteria that are known to produce antibiotics. We collected a soil sample on University of Northwestern - St. Paul property and diluted in a saline solution. The sample was plated on agar plates and 24 bacterial colonies were isolated. To test for antibiotic production, these 24 bacterial colonies were plated on three different plates spread with *E. coli*, *P. putida*, and *S. epidermidis*, which are safe relatives of the ESKAPE pathogens *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. One isolate was antibiotic producing, inhibiting the growth of all three safe relatives. To identify the isolate, gram-staining, biochemical tests, and 16S rRNA gene sequencing was done. It was found to be a 99% match to bacteria of the genus *Pseudomonas*. Further testing will be done on this isolate to examine the biologically active compound through a process called bioassay-guided isolation. This process will utilize a biological assay and purify the active molecule using liquid chromatography and obtain a chemical formula by using mass spectrometry.

SHIFTING THE BALANCE IN FAVOR OF ANTI-INFLAMMATORY MICROGLIA/MACROPHAGES IN ISCHEMIC BRAIN INJURY WITH NH-UCBSC AND IL-13 TREATMENT

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Microglia and macrophages (M/M) play essential roles in neuroinflammation, particularly after ischemic stroke. In response to ischemia, nonhematopoietic umbilical cord blood stem cell (nh-UCBSC) administration treatment is promising for reducing stroke infarct volume and improving functional recovery. IL-13, an anti-inflammatory cytokine, has been studied in stroke models as an agent in facilitating alternative activation of anti-inflammatory phenotype (M2) microglia/macrophages. I hypothesized that a nh-UCBSCs and IL-13 combined treatment will improve stroke outcomes by prolonging the phenotypic switch from anti-inflammatory to pro-inflammatory (M2-to-M1), characteristic of the neural microenvironment at 7 d post-stroke. Using the middle cerebral arterial occlusion model in Wistar rats, nh-UCBSCs were administered by intracerebroventricular injection into the lateral ventricles 48 h following stroke, with IL-13 presently administered. Preliminary results support nh-UCBSC + IL-13 therapy in addressing the pro-inflammatory shift and increased M/M recruitment to the site of injury. We believe that low cytometry analysis of immune cells in sham ischemic animals, animals without treatment, animals with nh-UCBSC, or IL-13 treatment alone, and combined treatment, will reveal a restoration of microglia to pre-infarct levels and confirm macrophage and T cell infiltration. Behavioral, histological, and immunohistochemical analyses are expected to show that nh-UCBSC administration will reduce infarct volume by 50%, and IL-13, by nearly 34%, both treatments being effective in ameliorating neurological deficits. Together, it is proposed that nh-UCBSC and IL-13 treatments will work in conjunction to maintain a longer-lasting M2-dominated, protective phase and prolong the detrimental M2-to-M1 shift. This combined therapy represents a potential immunotherapy in promoting long-term ischemic stroke recovery.

DISCOVERY AND ANALYSIS OF ANTIBIOTIC PRODUCING *Streptomyces* FROM SOIL

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As antimicrobial resistance to various current antibiotics continues to increase, the number of deaths from infections caused by antimicrobial resistant pathogens could grow to 10 million deaths per year by 2050. Some of the greatest antibiotic resistance is found in bacteria known as ESKAPE pathogens which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. The Tiny Earth Network offers students the opportunity to discover new potential antibiotics by searching for novel antibiotic producing bacteria from the soil that hinder the ESKAPE pathogen safe relatives. We hypothesized that soil contains potential antibiotic producing bacteria since several antibiotics originated from bacteria found in the soil. We collected soil from Arden Hills, Minnesota. The soil sample was diluted and plated on Brain Heart Infusion plates. Of the soil isolates screened for antibiotic production, one of the isolates showed zones of inhibition against the ESKAPE safe relatives *B. subtilis* and *S. epidermidis*. The soil isolate was then analyzed further through various biochemical tests and a genomic analysis of a portion of the 16S rRNA gene. We concluded from the bioinformatic analysis of the 16S rRNA sequencing results that the soil isolate was from the genus *Streptomyces*. The results support the hypothesis since the genus *Streptomyces* is predominantly found in the soil and is the largest source of antibiotics. Further research will analyze the isolate's resistance to any common antibiotics, and if the antibiotic produced is a novel antimicrobial compound.

CAPTIVITY CONVERGES THE MICROBIOMES OF DIVERSE PRIMATE SPECIES

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While associations between gut microbiome composition, host environment, and host health have been broadly documented, many details of these relationships remain unclear. By analyzing both cross-sectional and longitudinal fecal samples from a diverse group of captive non-human primates (NHPs) from a single zoo, we aimed to further explore the ways in which captivity influences the NHP gut microbiome. All samples were processed using both "open" and "closed reference" pipelines for comparison. For both longitudinal and cross-sectional samples, both pipelines returned significantly consistent outputs, indicating that analysis method is unlikely to substantially bias results. Within the cross-sectional cohort, we found that host species was the clearest driver of microbiome composition among captive NHPs. However, when comparing captive and wild individuals from similar species, captivity status had a greater influence on microbiome composition than host species. Additionally, historic antibiotic usage was linked to a pattern of decreased alpha diversity in captive emperor tamarins (*Saguinus imperator*). Preliminary analysis of the longitudinal cohort, which encompasses the relocation of two golden-headed lion tamarins (*Leontopithecus chrysomelas*) to the zoo, shows a similar pattern of decreased alpha diversity during and immediately following antibiotic usage in one individual. Furthermore, the integration process was linked to substantial changes in the gut microbiome, including the convergence of gut microbiome composition in newly cohabiting individuals.

IDENTIFICATION OF DRUGS POTENTIALLY INVOLVED IN PLANARIAN REGENERATION FROM A SCREEN OF A DRUG-GENE INTERACTION DATABASE

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Freshwater planarian flatworms have served as a classical model system for developmental and regenerative studies. Many genes underlying planarian regeneration have been identified from the application of RNA-

mediated genetic interference. However, the molecular and cellular mechanisms in which these genes are involved are not fully understood. We screened 40 genes required in planarian regeneration for drug-gene interactions (DGIs) through DrugCentral, an online drug compendium. Over 350 compounds were identified to modify these 40 genes. Among them, only a small subset has been shown in the literature to modify planarian regeneration. Collectively, these findings provide a novel extensive list of DGIs that could be investigated in future studies to illuminate the relationship between genes, chemicals, and phenotypes displayed in planarians. Preliminary studies on the effect of these drugs in worm regeneration are in progress. Understanding these effects will provide greater insights into the mechanisms of these genes, which would have significant implications in developmental biology and regenerative biomedicine.

CHEMISTRY

REACTIVITY OF VINYL HYDROPEROXIDES AND NEW REARRANGEMENT PATHWAYS IN ISOPRENE OZONOLYSIS

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Isoprene ozonolysis is the reactive process between ozone and isoprene – the most abundant alkene in the troposphere. This process generates many different products, a significant portion of which are known to be hazardous to human health and harmful to the environment. Though the ozonolysis of alkenes has been well studied, the specific mechanisms of isoprene ozonolysis have not been completely reported in the chemical literature. Given the need to understand the reactions that generate the aforementioned dangerous products, our primary research goal was to construct mechanisms and identify intermediates in the process of isoprene ozonolysis. It was necessary to use computational methods to study these reactions due to the very high energy of the transition structures; their instability causes them to decompose extremely quickly, preventing researchers from being able to effectively measure them experimentally. Isoprene ozonolysis involves the initial formation of a primary ozonide whose cycloreversion leads to Criegee intermediates. The syn conformer of a Criegee intermediate preferentially undergo an intramolecular 1,4-hydrogen shift to form an unsaturated vinyl hydroperoxide, which can either fall apart to give a hydroxyl radical or undergo a 1,3-shift to the hydroxyl group to form hydroxy acetaldehyde. Given the possibility of the radical pair rearrangement instead of falling apart, our research goal was to consider the rearrangement pathways for vinoxy and hydroxyl radicals that result from isoprene ozonolysis as well as comparing reactivity of vinyl hydroperoxides.

SYNTHESIS AND IN-SILICO ANALYSIS OF AZO-DYE INHIBITORS OF LOW MOLECULAR WEIGHT PROTEIN TYROSINE PHOSPHATASE

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Low molecular weight protein tyrosine phosphatase (LMW-PTP) is an enzyme that acts on many phosphotyrosine-containing cellular proteins involved in signal transduction, including aberrant growth factor signaling. Recent studies have assessed the role of LMW-PTPs in malignant cell transformation and have shown that the expression of LMW-PTP mRNA and protein is significantly increased in human breast, colon, bladder, and kidney tumor samples. Moreover, its enhanced expression is generally prognostic of a more aggressive cancer and reduced survival rate. Small molecule inhibitors of LMW-PTP have been investigated for their anti-cancer properties by inhibiting dephosphorylation of certain receptors. We synthesized twenty-three azo dye analogs of (4Z)-4-[[4-(carboxymethyl)phenyl]hydrazono]-3-keto-2-naphthoic acid, a lead compound from a previous study in our lab, and we examined their binding to LMW-PTP isoform B in silico. Multiple potential inhibitors

demonstrated promising computational affinities, indicative of therapeutic potential which will eventually be further examined using in-vitro assays.

DEHYDRATION OF 4-HYDROXY-4-METHYLPENTAN-2-ONE USING AN ECO-FRIENDLY CATALYST

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In this experiment, we performed a unimolecular elimination (E1) reaction on 4-hydroxy-4-methylpentan-2-one, using K10 Montmorillonite catalyst, to form 4-methylpent-3-en-2-one. The reason 4-hydroxy-4-methylpentan-2-one was chosen as the substrate, was due to the presence of the ketone and alcohol function groups. The ketone group was necessary so that after the reaction both the starting material and the product would be polar molecules, allowing them to be detected by the Vernier Mini Gas Chromatograph. K10 is a layered aluminasilicate clay which is inexpensive and environmentally friendly. One drawback of K10 is that it also catalyzes a side reaction that produces two molecules of acetone, therefore, acetone is produced along with the product. However, the acetone formed can reform the starting material which can then go on to irreversibly form the desired product. The reagents were allowed to react at varying times ranging from 30 min to 75 min. After a given time the reaction mixture was distilled and gas chromatography (GC) was used to analyze the outcome of the reaction. GC results indicated that the K10 Montmorillonite was successful in catalyzing the reaction. According to the GC results there appears to be a direct correlation of reaction time to product yield with 30 min reaction time showing the lowest amount of product and the 75 min reaction, showing the most product. K10 appears to be a quality substitute for the acid catalysts normally used in alcohol dehydration. Although K10 catalyzes the formation of acetone this can be overcome by increasing reaction time.

PHOSPHATE CONCENTRATION ANALYSIS IN MUNICIPAL DRINKING WATER RESERVOIRS

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With recovery from the water crisis in Flint, Michigan still underway, municipal water safety has been recently highlighted. The decision to switch municipal water sources resulted in drastically changed biological and chemical profiles of municipal water across a broad spectrum of categories. Adequate adjustments to neutralize dangerous components in the new water source were neglected, resulting in the release of unsafe levels of harmful lead into Flint's water supply. One method of safeguarding against such contamination is to add a degradation inhibitor, called orthophosphate, into a water supply. When introduced at appropriate levels, orthophosphate binds with lead which forms a protective layer on pipes. This is called a passivation layer. Analysis of municipal water sources is important to ensure water safety. While this process is multi-faceted, a key component of analysis is testing for appropriate levels of phosphate. We collected water samples from municipal water sources in southeastern Minnesota via water tap. Samples were taken at first draw. We determined phosphate concentration via spectrophotometry. We created a concentration curve from known concentrations of phosphate and with distilled water as the control. We compared readings from the municipal water samples to the concentration curve to determine municipal water phosphate concentrations."

ECOLOGY AND ENVIRONMENTAL SCIENCE

Echinacea angustifolia SEEDLING PERSISTENCE DOES NOT VARY WITH MICROHABITAT CHARACTERISTICS OR FLORAL NEIGHBORHOOD IN FRAGMENTED PRAIRIE

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Habitat fragmentation and anthropogenic land-use change threaten prairie ecosystems, and long-lived, self-incompatible perennials like *Echinacea angustifolia* are at risk of population decline under these conditions. Seedling establishment and juvenile persistence are key aspects of plant population dynamics and little is known about how these processes occur in fragmented *E. angustifolia* populations. In this study we investigated whether microhabitat characteristics affect juvenile persistence in fragmented *E. angustifolia* populations in rural western Minnesota. During summer 2020 we collected microhabitat data on 69 surviving juvenile *Echinacea* plants that established between 2007-2013, along with a random sample of 66 locations where an *Echinacea* juvenile had established during the same period but had since died. Microhabitat data was collected in a 1-meter radius surrounding each surviving or former seedling, and included number of flowering species and inflorescence counts, distance to nearest road and/or field, vegetation cover, litter depth, slope, and aspect. Using multivariate analysis, we found no significant differences between the living and dead groups in any of the microhabitat characteristics measured, and in addition, found no differences in microhabitat characteristics among prairie fragments. This suggests that the microhabitat characteristics measured are not strong determinants of juvenile survival in fragmented *Echinacea* populations, and that future research should focus on other factors, such as annual climatic variation, soil nutrients and moisture, and genetics, all of which may contribute to *Echinacea* juvenile survival and establishment.

LEAF RADIOCARBON AND REMOTE SENSING REVEAL LEGACIES OF REDLINING IN MIDWESTERN CITIES

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Redlining broadly refers to the racist, classist, and anti-immigrant policies of home-loan lending in cities during the 1930-1940s by the Home Owners Loan Commission (HOLC). While no longer legal, this practice can be traced to current-day environmental injustice and inequity. Using a hybrid-methods approach of radiocarbon isotope ratios and remote sensing, we asked the following questions: (1) can leaves of trees and annual plants integrate a signal of fossil fuel emissions that reflect differences amongst neighborhoods that were historically 'redlined' and 'greenlined'?; and (2) can differences in air pollutant concentrations among these neighborhoods be detected remotely using satellite imagery? Using St. Paul, MN, USA as a pilot site, we collected leaves from plants in different HOLC neighborhoods (n=12), as well as a remote, non-urban site. Radiocarbon isotope data of these leaves indicate the proportion of fossil-fuel derived carbon assimilated within the 2020 growing season, providing a local signal of CO_2 emissions. We then expanded our study to include ten US Midwestern cities to evaluate current concentrations of NO_2, CO, and ozone gasses using TROPOMI satellite data. Our data indicate plant leaves integrate a local fossil fuel emission signal. No difference in fossil fuel emissions was found between HOLC grades, but concentrations of NO_2, CO, and Ozone among and within cities still point to ongoing inequity in air quality. Together, these data present a novel hybrid-methods approach to examining the legacy of redlining on the air pollution exposure of urban populations.

EFFECTS OF GOAT GRAZING INTENDED FOR *Rhamnus cathartica* ON SURROUNDING FORBS AND NATIVE WOODY PLANT SPECIES ON BETHEL UNIVERSITY'S CAMPUS

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Invasive species persist as a continuous threat to the biodiversity of many ecosystems today. In an effort to remove the invasive species European buckthorn, *Rhamnus cathartica*, from Bethel University's campus, we implemented targeted goat grazing for two consecutive years. We examined how the native forbs and woody non-buckthorn plant species were impacted alongside *R. cathartica* as these grazing treatments took place. The primary purpose of this project was to analyze if two consecutive years of goat grazing had a statistically significant impact on both the number of forbs and woody non-buckthorn plant species. For data collection, we laid out forty six 1x1 meter sample plots established within grazed (n=26) and ungrazed (n=20) areas where plants were categorized and counted. Grazing had a statistically significant impact on both the number of forbs and woody non-buckthorn plants within the treated areas, and there was no significant change in the numbers of these plants in the ungrazed areas.

SEASONAL CHANGES IN BLACK-CAPPED CHICKADEE FORAGING AND BEHAVIORAL PATTERNS

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The northern Midwest region of the United States experiences substantial changes in weather patterns throughout the year. For year-round resident songbirds, survival during the harsh winter months depends on their ability to adapt, both behaviorally and physiologically. Successful foraging is especially critical during this period, which is likely to involve changes in foraging behavior in response to seasonal variation in food availability. For a period of approximately seven weeks in the fall of 2020 and another of six in the early spring of 2021, we studied the foraging behavior of Black-capped Chickadees (*Poecile atricapillus*) on the University of Northwestern campus in east-central Minnesota to gain a greater understanding of how the birds modify both their foraging and general behavioral patterns with these seasonal changes. We recorded their various behaviors using time activity budgets. We then compared them to ascertain whether any statistically significant changes in foraging behavior occurred either intra-seasonally or between fall and spring. Altogether, we conducted 28 observational sessions in the fall, yielding a total of more than 72 minutes of activity. Initial analysis of these data points to the possibility of statistically significant changes in some foraging categories. Collection and analysis of spring data is still ongoing.

CALL CHARACTERISTICS OF SOUTHERN FLYING SQUIRREL (Glaucomys volans) VOCALIZATIONS IN MISSOURI

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Vocalizations in animals are used for communication, mating, hunting, and predator avoidance. The forms these vocalizations take vary from species to species. Vocalizations can also vary within a species based on gender, age, social status, and geographic location. Geographic variation occurs when populations of the same species are geographically isolated from each other. Most animal vocalizations are audible to humans, unlike ultrasonic sound which is any sound that is above 20 kHz. Since its discovery in 1793, studies of ultrasound have focused on the echolocating abilities of bats and whales. However, recent studies have shown that other mammalian taxa use ultrasonic sound especially those in the order of Rodentia. It has been recently discovered that southern flying squirrels (*Glaucomys volans*) use ultrasonic vocalizations and these vocalizations can differ based on geographic

location. To characterize the calls of southern flying squirrels, we analyzed the recorded calls of wild southern flying squirrels from Missouri. Frequency and time parameters were measured for the recorded calls and cluster analysis was used to group like call syllables together. Our analysis produced 7 different clusters or call syllables. Some call syllables appeared to match known syllable types and others did not match anything. This shows that southern flying squirrel calls are complex and different call syllables may have different meanings. These meanings may differ depending on the receiver individual or on the situation taking place. Future studies should look at how and when call syllables are being used by southern flying squirrels to better understand their vocal repertoire.

ACCUMULATION OF 17α-ETHINYLESTRADIOL IN POOL 6 OF THE MISSISSIPPI RIVER

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Human synthetic hormone use has varied environmental impacts, and recent studies have shown that low concentrations of synthetic hormones are problematic for aquatic life. Synthetic estrogens, like 17α-Ethynylestradiol (EE2), act as endocrine disruptors in aquatic species. When EE2 enters the bodies of fish and other aquatic organisms, it has been shown to feminize males of the species, leading to lowered mating capability and hence lower populations. We investigated whether the water treatment plant in Winona, MN is a source of synthetic estrogens in the Mississippi River, and how those estrogens may be accumulating at greater depths downstream of the water treatment plant. We found no significant difference in the EE2 concentration above or below the water treatment plant , ($n=12$, $p=0.200$, $p=0.700$), which is the expected source of synthetic estrogens in effluent. We also found no significant difference between the concentration of EE2 in water samples from depths of 20 and 150 cm ($n=12$, $p=0.100$, $p=0.700$). However, there was a significant difference in the concentration found between the samples obtained from Pool 6 and the control, Lake Winona. This demonstrates that there are low levels of synthetic estrogens in the water from Pool 6. Building off the methods presented in this paper and through larger sample sizes, future research will be able to further examine these relationships.

ENGINEERING

CALIBRATING OPTICAL PARTICLE COUNTERS TO EXAMINING HYPERSONIC BOUNDARY LAYER TRANSITION THROUGH HIGH ALTITUDE BALLOONING

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When the hypersonic boundary layer on an aircraft moving at several times the speed of sound transitions from smooth laminar flow to chaotic turbulent flow, the skin friction and aerodynamic heating rate dramatically increase. Thus, the design of hypersonic vehicles relies on the ability to predict laminar-to-turbulent transition. It is currently not known if atmospheric turbulence or suspended particulates are the main sources of the disturbances that cause this transition. Therefore, it is important to characterize the atmospheric state at stratospheric altitudes where hypersonic vehicles fly. To study this problem, the Air Force Office of Scientific Research is funding a Multidisciplinary University Research Initiative (MURI) comprising of the University of Colorado - Boulder, Embry Riddle Aeronautical University in Florida, and the University of Minnesota - Twin Cities. The University of Minnesota's role is to characterize particulate concentration in the stratosphere utilizing weather balloons carrying particle-measuring instruments. The MURI Ballooning Team works to test and modify optical particle counters (OPCs) to fly on stratospheric balloon flights. The team has also constructed a calibration chamber in which known micron-size particles can be fed into OPCs being subjected to the extremely low temperature and low-pressure conditions of the stratosphere. This chamber is used to recalibrate low-cost OPCs

for use in stratospheric conditions. Ultimately, utilizing both high-altitude ballooning and ground-tested OPCs in the calibration chamber will assist in determining how the presence of atmospheric particulates influences the transition of hypersonic boundary layers from laminar to turbulent.

NEUROSCIENCE

INFLUENCE OF HEAVY ALCOHOL EXPOSURE ON THE CHRONIC PAIN MECHANISM

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Alcohol use disorder (AUD) and pain are separate neurophysiological conditions that, when chronically and comorbidly present in patients, serve to disrupt normal daily functioning. The comorbidity of AUD and pain suggests there is overlap in neuronal activation when both conditions are present. Neurons activated during the reward and aversion aspects of chronic alcohol use may similarly be activated during chronic and acute pain. Though neural circuits have been separately identified, much less is known about the mechanisms of AUD in comparison with other sensory modalities such as pain. Since little is known about the overlapping neural circuitry of AUD and pain, this study plans to use the TRAP method to identify activated neurons expressing the fluorescent reporter protein tdTomato in a time-dependent manner under different experimental conditions of alcohol consumption and pain. After TRAP, immunohistochemistry will assess anatomical similarities between AUD and pain by identifying neuronal activation under each condition. We will use mechanical hypersensitivity testing to observe if chronic alcohol consumption exacerbates persistent pain. Prior to studying the interaction of alcohol and pain, we validated the efficacy of the TRAP method by examining neural circuits activated by pain. Brain regions that were activated by these stimuli included S1/S2, anterior cingulate cortex, and insular cortex. Expected overlapping brain regions for AUD and pain include mPFC, NAc, EW-nucleus, and amygdala. Unveiling overlaps in neural activity related to chronic alcohol use and pain will set a foundation for functional work that uncovers clinical treatments for the comorbidity of AUD and pain.

ORGANISMAL AND PHYSIOLOGICAL SCIENCES

AN ANALYSIS OF DESICCATION TOLERANCE OF THE FERN *Polypodium virginianum*

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Desiccation tolerant (DT) plants can equilibrate their internal water potential with the surrounding air without incurring significant damage. Oftentimes this means losing more than 90% of their water content and still recovering normal function after rehydration. For a plant to survive this severe water loss, it must have mechanisms to counteract the mechanical and photooxidative stress that desiccation causes. No major agricultural plants possess this tolerance. Most DT species are small, nonvascular plants such as lichen, algae, and moss; however, a small percentage of ferns possess this unique capability as well. My research investigated the level at which the fern *Polypodium virginianum* is desiccation tolerant. I found that it can survive up to 90% water loss, but that it struggles to recover under truly desiccated conditions. Due to this distinction, I propose that it is defined as a desiccation sensitive plant, which still means it is very well adapted to surviving water loss. This presents a unique refinement of the classification of *P. virginianum*. Additionally, I examined the role of the xanthophyll cycle pigments in aiding with DT. The xanthophyll cycle consists of three pigments (violaxanthin, antheraxanthin

and zeaxanthin) that are alternately upregulated to handle the plant's changing environment. The pigment zeaxanthin aids in preventing photooxidative stress that can result from water loss. My research found that zeaxanthin was significantly upregulated during the desiccation and was de-epoxidized back into violaxanthin once rehydration occurred. This supports the consensus that zeaxanthin is a key player in the response to extreme water stress.

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